NanoPhotometer NP80/N60/N50/C40

User Manual

Version 1.0.5

Declaration of conformity for the NanoPhotometer (C40/N50/N60/NP80)

This is to certify that the Implen NanoPhotometer conforms to the requirements of the following directives:

2014/35/EU	Low Voltage Equipment Safety Directive
2004/108/EC	EMC Directive
IEC 60529	Protection class IP20
2002/95/EC	Restrictions on the use of certain Hazardous Substances in Electrical and Electronic Equipment (ROHS)
2012/19/EU	EC Directive on Waste Electrical and Electronic Equipment (WEEE) 2003/108/EC & 2008/34/EC. By ensuring this product is disposed of correctly, you will help prevent potential negative consequences for the environment and human health, which could otherwise be caused by inappropriate waste handling of this product.

FCC 47 CFR Part15 §15.107 and §15.109

EN 301 489-1 V1.9.2	Radio and ancillary equipment for portable use (portable equipment); EUT Operating frequency range: 2.4 – 2.4835 GHz
EN 301 489-17 V2.2.1	Electromagnetic compatibility and Radio Spectrum Matters (ERM)
IEC 62133 and UN38.3	Battery certification and transport test

Standards to which conformity is declared, where relent, are as follows:

IEC/EN 61010-1:2012	Safety requirements for electrical equipment for measurement, control and laboratory use. General requirements.
EN61326-1:2013	Electromagnetic compatibility- generic emission standard electrical equipment for measurement, control and laboratory use.

Signed:

Dr. Thomas Sahiri
Managing Director
Implen GmbH

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1. NanoPhotometer at a Glance

NanoPhotometer Overview

The Implen NanoPhotometer spectrophotometer is a mobile, simple to use UV/Visible instrument with a CCD array detector with options ranging from nanovolume to standard cuvette and all-in-one solutions. The N50, N60, and NP80 spectrophotometers measure sample volumes ranging from 0.3 - 2 µl. The NP80 model also has the capability to measure samples using a standard cuvette. The C40 is a mobile standard cuvette solution with the option to upgrade to accommodate nanovolume measurements.

The NanoPhotometer runs on a Linux based operating system (NPOS) that is designed for the use of pre-programmed and custom applications with a high degree of flexibility and processing power.

Sample Compression Technology™ provides easy sample handling which is independent of surface tension. This technology squeezes the sample between two quartz surfaces allowing for unmatched precision and accuracy without the need for dilutions. Combined with our True Path Technology™ the system offers lifetime accuracy and precision without the need for maintenance or recalibration.

Note: It is recommended to use a properly calibrated pipette with high quality tips to ensure delivery of appropriate sample volumes for nanovolume sample applications.

NANOPHOTOMETER NP80

Item Number	Touchscreen	Battery Pack
NP80	-	-
NP80-Touch	+	-
NP80-Mobile	+	+

NANOPHOTOMETER N60

Item Number	Touchscreen	Battery Pack
N60	-	-
N60-Touch	+	-
N60-Mobile	+	+

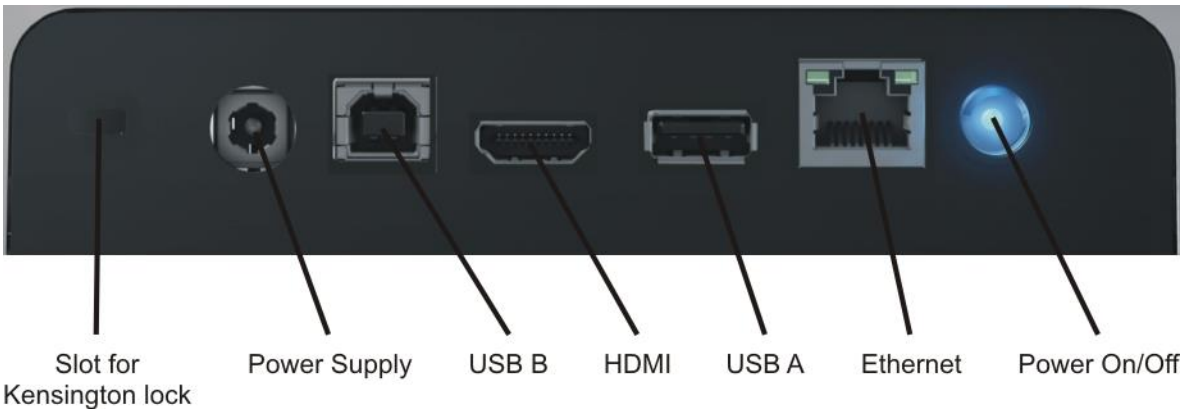
NANOPHOTOMETER N50

Item Number	Touchscreen	Battery Pack
N50	-	-
N50-Touch	+	-

NANOPHOTOMETER C40

Item Number	Touchscreen	Battery Pack
C40	-	-
C40-Touch	+	-
C40-Mobile	+	+

Instrument Rear Panel (NP80/N60/N50/C40)



Instrument Bottom View (NP80/N60/N50/C40)

IMPLEN GMBH
Model: NanoPhotometer NP80 Mobile
Serial No.: M80701
Input: 19V ⎓ 4,7 A
SGS
C US
710281
This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions:
(1) this device may not cause harmful interference, and
(2) this device must accept any interference received, including interference that may cause undesired operation.
IMPLEN
Made in Germany
CE
FC
Contains FCC ID: NDD9578111006
Contains IC ID: 4701A-78111306

Model name, device serial number and FCC ID are located on the identification plate on the bottom of the instrument.

ACCESSORIES

STANDARD ACCESSORIES

- **Connecting Cable**

The USB cable provided makes it possible to connect the NanoPhotometer to a computer to control the instrument from the computer (please see Software Installation).

- **NanoPhotometer Power Adapter**

Use the power adapter to plug in the NanoPhotometer for use and/or charge the NanoPhotometer battery (touch and mobile models with optional integrated battery).

Note: Use only the power adapter, supplied with your instrument or a replacement part from the manufacturer or your supplier.

- **Dust Cover**

OPTIONAL ACCESSORIES

- **Didymium Glass Filter (C40/NP80)**

The certified didymium glass filter is used as a control of wavelength and photometric accuracy for the NanoPhotometer cuvette applications.

- **Submicroliter Cell (C40 only)**

The submicroliter cell provides the option of upgrading the NanoPhotometer C40 to a device that handels nanovolume samples as well as cuvette samples. The sample volume range of the submicroliter cell ranges from a minimum of 0.3 µl to 5.0 µl with the path length options including: 0.04 mm, 0.1 mm, 0.2 mm, 1 mm and 2 mm which correspond to virtual dilution factors of 1:250, 1:100, 1:50, 1:10, and 1:5 respectively.

- **DiluCell (C40/NP80 only)**

The DiluCell is a disposable cuvette with shortened path length for virtual dilution of cuvette based samples. Due to the reduced path length the DiluCell provides an automatic dilution without the need of a physical dilution of higher concentration samples. There are two different sizes of DiluCells available DC 10 and DC 20 which allow an automatic 1/10 and 1/20 dilution of the sample respectively. Bypassing manual sample dilutions reduces dilution errors and cross contamination making DiluCell ideal for GLP. Combined with small sample volume requirements and bubble free filling, the Dilucell allows for convenient spectrophotometric analysis from 340 - 950 nm.

- **Field Kit**

Implen aluminium roller case for safe transportation, designed to fit in most airplane overhead bins; special compartments for all necessary accessories, cleaning tools and samples.

Note: Delivered w/o NanoPhotometer and accessories
Note: Only operate the NanoPhotometer in the roller case when cover is open. Ensure that air circulation is possible. Switch the NanoPhotometer always off for transportation.

CONNECTIVITY

- **WiFi**

The NanoPhotometer has a WiFi hotspot capability. This provides the option to control the NanoPhotometer by other WiFi devices like smart phones or tablets.
SSID: NanoPhotometer serial number; password: Implenuser

- **USB A**

There is a USB A port on the front and rear panel of the NanoPhotometer which is compatible with standard portable USB storage devices for direct data transfer in a variety of formats including Excel. It is also possible to connect a mouse or keyboard directly to the NanoPhotometer.

- **USB B**

There is a USB B port located on the rear panel of the instrument which is compatible with the USB cord provided to connect the NanoPhotometer to a computer. This USB connection can be used to control the NanoPhotometer via a computer.

- **LAN**

There is an Ethernet (LAN) connection port on the rear panel of the instrument which enables the NanoPhotometer to connect with computers within close proximity.. This Ethernet connection can be used to transfer data from the NanoPhotometer to a computer. To access the directory of the NanoPhotometer via LAN enter the serial number/IP of the NanoPhotometer in the Windows-Explorer/Mac OS X Finder and confirm with the Enter key.
Note: The maximal LAN cable length is 10 meters. Bit rate is 1 Gbit/s.

- **HDMI**

There is an HDMI port located on the rear panel of the NanoPhotometer which is compatible with HDMI 1.4 (or better) cables to connect the NanoPhotometer to HDMI compatible monitors.

NANOPHOTOMETER SPECIFICATIONS

NanoVolume Performance

Detection Range dsDNA	1 ng/µl to 16,500 ng/µl (N50: 5 ng/µl to 7,500 ng/µl)
Detection Range BSA	0.03 mg/ml to 478 mg/ml (N50: 0.15 mg/ml to 217 mg/ml)
Minimum Sample Size	0.3 µl
Photometric Range (10 mm equivalent)	0.02 - 330 A (N50: 0.1 – 150A)
Path length	0.67 and 0.07 mm
dilution factor	15 and 140
Vortexer	2,800 rpm; tube size up to 2.0 ml

Cuvette Performance

Detection Range dsDNA	0.1 ng / µl to 130 ng / µl
Detection Range BSA	0.003 mg / ml to 3.7 mg / ml
Photometric Range	0 - 2.6 A
Center Height (Z-Height)	8.5 mm
Cell Types	outside dimension 12.5 x 12.5 mm
Heating	37°C ± 0.5°C

Optical Specifications

Wavelength Scan Range	200 – 900 nm (N50: 200 - 650 nm)
Time for Full Scan Range	3.5 – 6.0 seconds
Wavelength Reproducibility	± 0.2 nm (N50: ± 1nm)
Wavelength Accuracy	± 0.75 nm (N50: 1.5 nm)
Bandwidth	1.8 nm (N50: 5 nm)
Stray Light	< 0.5% at 240 nm using NaI (N50: < 2%) and < 1% at 280 nm using Acetone (N50: < 2%)
Absorbance Reproducibility	< 0.002 A (0.67 mm path) @ 260 nm (N50: < 0.004 A (0.67 mm path) @ 260 nm)
Absorbance Accuracy	< 1.75 % @ 0.7 A (0.67 mm path) @ 260 nm of the reading
Zero Stability	±0.003 A/hour after 20 min warm up @ 260 nm
Noise	0.002 A rms at 0 A @ 260 nm; 0.002 A (pk to pk) at 0 A @ 260 nm
Optical Arrangement	1 x 3648 CCD Array (N50: 1 x 1024 CCD Array)
Lamp	Xenon flash lamp
Lifetime	10^9 flashes, up to 10 years

Processing Power and Compatibility

Operating System	Linux based OS
Onboard Processor	Quad Core 1 GHz
Internal Storage	8 GB
Control Options	Onboard with built-in touchscreen, computer, smart phone and tablet
Software Compatibility	Windows 7 (32 and 64 bit), Windows 8 (32 and 64 bit), OS X, iOS and Android
Min. Requirement Smart phone/Tablet	4 inch screen; Apple: iPad 2, iPhone5 and iOS 6 Android version 2.2, Quadcore 1.2 GHz with 1 GB RAM

General Specifications

Size	200 mm x 200 mm x 140 mm
Weight	3.8 – 5.2 kg depending on configuration
Operating Voltage	90-250 V, 50/60 Hz, 60 W (90 W with battery pack), 18/19 VDC
Display	1024 x 600 pixels; touchscreen glove compatible
Certifications	CE, IEC/EN 61010-1:2012 and EN 61326-1:2013
Battery Pack	optional rechargeable lithium ion battery; 95Wh, 6,6Ah; Operation time: up to 8 h; min. charging cycles: 800
Battery Certification	IEC 62133 and UN38.3 transport test
In- and Output Ports	2x USB A, USB B, HDMI, Ethernet, WLAN
Additional Data Input	Mouse and keyboard options
Security	Slot for Kensington lock

Features and specifications are subject to change without notice.
US Patents 20080204755 and 20080106742
Windows is a trademark of Microsoft. Mac OS & iOS are trademarks of Apple. Android OS is a trademark of Google. Linux is a trademark of Linus Torvalds.

2. GETTING STARTED

SPECTROPHOTOMETER INSTALLATION

- **Safety Information**

Before commencing installation, please take time to familiarize yourself with warning labels and symbols on your instrument and their meaning. These are to inform you where potential danger exists or particular caution is required.

⎓ direct current

Do not open the instrument as this can expose the operator to electrical power, UV light and delicate fiber optics.

Do not use damaged power cords, accessories, and other peripherals with your NanoPhotometer.

Do not expose the NanoPhotometer to strong magnetic or electrical fields.

Do not leave your NanoPhotometer on your lap or near any part of your body to prevent discomfort or injury from heat exposure.

Do not place objects on top of your NanoPhotometer.

The NanoPhotometer with battery pack (mobile version) has to be switched off during transportation. The on/off button has to be protected from turning on itself caused by shock or vibration.

The symbol on the product, or on the documents accompanying the product, indicates that this appliance may not be treated as household waste. Instead it shall be handed over to the applicable collection point for the recycling of electrical and electronic equipment. Disposal must be carried out in accordance with local environmental regulations for waste disposal.

Unpacking and Positioning

Check the contents of the package against the delivery note. If any shortages are discovered, inform your supplier immediately.

Inspect the instrument for any signs of damage caused in transit. If any damage is discovered, inform your supplier immediately.

Ensure your proposed installation site conforms to the environmental conditions for safe operation: indoor use or dry environment.
Note: Do not expose your NanoPhotometer near liquids, rain, moisture or dusty environments.

Temperature range 10 - 40 °C; If the cuvette heating is used the range is 10 - 30°C.

If the instrument is subjected to extreme temperature changes, it may be necessary to allow the instrument to equilibrate and to turn the instrument off and then on again once thermal equilibrium has been established (~2 - 3 hours).

Maximum relative humidity (non-condensing) of 80% and up to 31°C decreasing linearly to 50% at 40°C.

The instrument must be placed on a stable, level surface that can support 4 - 5 kg and so that air can circulate freely around the instrument. Ensure while powered on that no materials reduce air circulation.

The equipment should be positioned such that in the event of an emergency the main plug can be easily located and removed.

Carry the instrument always by holding the main corpus of the instrument and not e.g. on the optional attached display or NanoVolume pedestal.

The equipment must be connected to power with the 60W power supply/cord supplied by Implen (90W with battery pack). It can be used on 90 - 250 V, 50 - 60 Hz power supply system.

For Mobile models please load the battery pack at least for 3 hours prior to the first use.

Turn the instrument on using the power button on the rear panel after it has been plugged in. The instrument will perform a series of self-diagnostic checks.

Please read the complete user manual prior to use.

Please contact original supplier immediately if technical or sample handling difficulties are experienced.

Note: If this equipment is used in a manner not specified or in environmental condition not suitable for safe operation, the protection provided by the equipment may be impaired and the instrument warranty voided.

SOFTWARE INSTALLATION

NPOS OVERVIEW

NPOS is a Linux based operating system designed for the NanoPhotometer.

NPOS can store data either to a common directory or be configured to save to independent directories according to file format and/or instrument.

NPOS can save data in an Implen .IDS format or as an Excel format file.

Note: Please do not connect the instrument to a computer until the NanoPhotometer NPOS software is installed on the computer.

REQUIREMENTS AND COMPATIBILITY

The NPOS user interface is designed that all features can be operated by using a touchscreen. If the software is installed on a computer without touchscreen, the user interface can be operated by using a keyboard and a mouse. Before starting the installation process ensure the device is compatible with the requirements for installation.

Compatible Control Devices

Computer:
PC: Windows 7 / Windows 8 (32 bit or 64 bit)
Mac: OS X

Tablets:
iPad 2: iOS6
Android (Quadcore 1.2 GHz with 1 GB RAM): Android version 2.2

Smart phones:
iPhone 5: iOS6
Android (Quadcore 1.2 GHz with 1 GB RAM): Android version 2.2

Windows is a trademark of Microsoft. Mac OS & iOS are trademarks of Apple. Android OS is a trademark of Google. Linux is a trademark of Linus Torvalds.

Note: There are two user interfaces of the software available; one for built-in touchscreen, computer and tablets and one for smart phones.

INSTALLING SOFTWARE ON COMPUTER

The NanoPhotometer software can be installed on compatible PC and Mac computer systems. Various operating systems and PC hardware may cause the set-up procedure to differ from that described here. This process is given as guidance only; it may need adaptation for other systems. To prevent loss of data it is important to ensure that connections are stable and not lost during data transmissions and ensure that the system has enough time to save transferred sample data.

1. To install the NanoPhotometer software the installer should be logged with full administration rights. If you have insufficient privileges, installation may fail. If in doubt consult your PC administrator.
2. Connect the instrument to the computer using the USB cable provided

Note: The Windows and Mac installation file is provided on the Implen USB flash drive which is included in the NanoPhotometer delivery and are available for free download in the download section of the Implen webpage.

Note: If there is Avira installed on your computer, it is recommended to switch of the browser safety. This may interfere with the NPOS running on your computer.

INSTALLING NANOPHOTOMETER APP ON TABLET OR SMART PHONE

The NanoPhotometer App can be installed as an application on tablets and smart phones with compatible Android and iOS operating systems. The NanoPhotometer App is available for free download in the app store (Apple Store and Google Store).

1. Download and install the NanoPhotometer App from the app store
2. Use the WiFi to connect the NanoPhotometer to the tablet or smart phone.
   SSID: Serial number, password: implenuser
3. Open the NanoPhotometer App
4. When connected via WiFi the NanoPhotometer will recognize the tablet as a remote control device and measurements can be initiated from the tablet or smart phone
5. Measurement results will be shown to the tablet or smart phone once taken

Note: In order to install the NanoPhotometer App on a tablet or smart phone, the device must have an established internet connection to access the app store for app download.

FIRST STEPS AND CONFIGURATION WIZARD

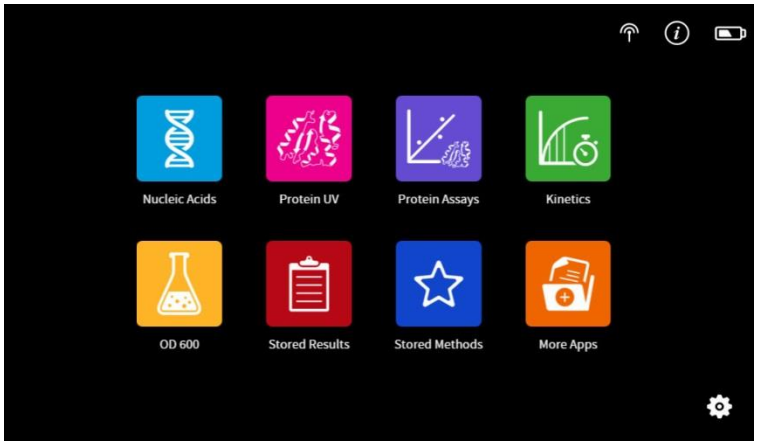
Starting the Implen NPOS the first time an Implen configuration wizard is shown. Please confirm the End User License Agreement (EULA) and select the country in which the NanoPhotometer is used and confirm.

3. NanoPhotometer Basics

The NanoPhotometer product line offers a complete solution for Nanovolume (NP80/N60/N50/C40) and standard cuvette volume (C40/NP80) applications. With the NanoPhotometer nanovolume applications the sample volume ranges from a minimum of 0.3 µl to a maximum sample volume of 2.0 µl. Standard cuvette applications can be performed with 10 mm, 5mm, 2 mm and 1 mm path length quartz, glass, or plastic cuvettes with a center height of 8.5 mm.

Applications Overview

The NanoPhotometer comes with pre-programmed applications as well as the ability to create custom applications. To select a method click/tap on the corresponding icon and the method will open immediately.



Method Icons	Description
Nucleic Acids	Concentration, purity, and dye incorporation for DNA, RNA, Oligo, and other nucleic acids
Protein UV	Protein UV determination at 280 nm (or in a range of 200 - 330 nm), purity and dye incorporation
Kinetics	Time vs. Absorbance readings
Protein Assays*	BCA (562 nm), Bradford (595 nm), Lowry (750 nm), and Biuret Assays (546 nm)
OD600*	Measures cell density at 600 nm (or in a range of 200 – 900 nm)

Stored Methods	Collection of stored custom methods
Stored Results	Archive of stored results
More Apps	Additional applications found on a secondary method screen

Wavelength	Define one or multiple wavelength between 200 – 950 nm (N50: 200 – 650 nm) for absorbance measurements
Wavescan	Define desired full scan range anywhere between 200 - 950 nm (N50: 200 - 650 nm)
Concentration	Define extinction coefficient for automatic concentration calculations
Absorbance/ Ratio	Define two wavelengths for a calculated absorbance/ ratio
Standard Curve	Create a standard curve at a defined wavelength
Custom Apps	Optional custom applications for personalized methods tailored to individual spectroscopy needs

Note: *For Protein Assays and OD600 applications the cuvette mode is recommended.

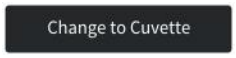
ICONS

Icon	Name	Action
	WiFi Hotspot	WiFi hotsopt enabeld; can be disabled in preferences
	Assistance	Opens the assistance page
	Battery Status	Shows the actual battery status (only shown with optional battery pack)
	Preferences	Opens the preferences page
	Home Screen	Returns to the home screen with application icons for method selection.
	Leave Method	Returns to the previous application selection
	Back	Returns to the previous page (smart phone only)
	Next/Confirm	Confirms parameter and opens the next screen (smart phone only)
	Delete	Deletes added functions in parameter; empties input windows
	Delete Data	Opens a delete dialog pop up
	Email Data	Opens an email dialog pop up (computer and tablet versions only)
	Save Data	Opens a save dialog pop up
	Add Folder	Adds a new folder to the directory
	Manage Data	Opens a dialog pop up with several action options including delete, rename or import folders/files/data as well as copying or move folders/files/data to defined directories
	Store Method	Opens a dialog pop up with the possibility to store the actual method parameter to a custom method
	Full Scale	Restores graph to original size without zoom
	Cancel	Returns to the previous screen without implementing any changes

BUTTONS

Prior to any sample measurement at the start of a new method a blank measurement of either water or the buffer of the samples is required to give the NanoPhotometer a reference of what zero should be. It is recommended to re-apply the blank and measure it as a sample to ensure the graph of the blank spectra is a flat line.
Note: Use always a minimum of 1 µl for any blank measurement.

To initiate the spectral scan of the sample press the sample button. The data will be temporarily stored until the method is exited at this time the user needs to define if the samples should be saved or deleted.



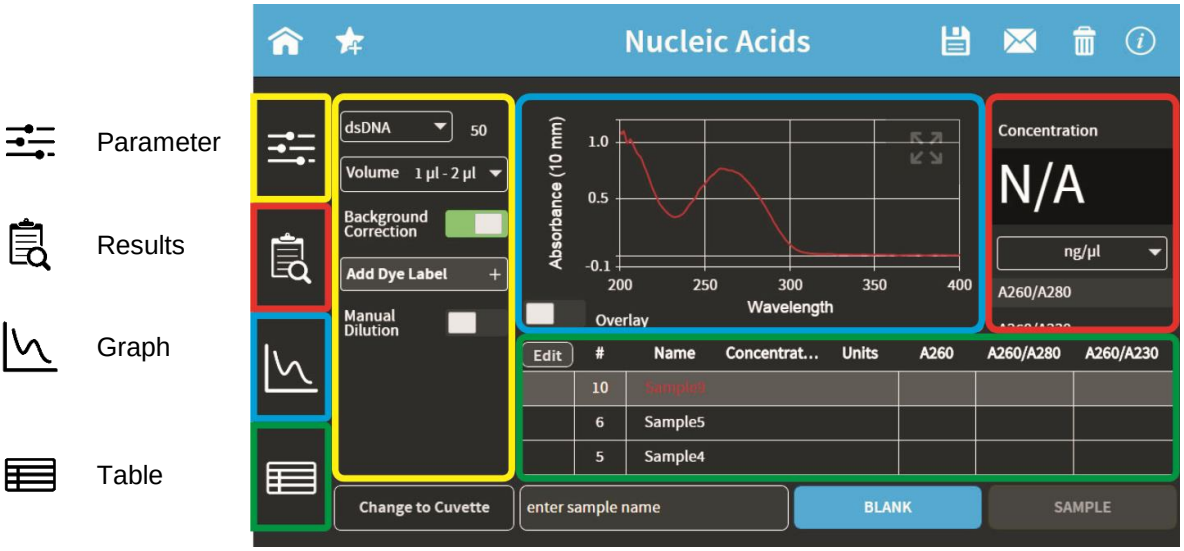
The Change to Cuvette button (NP80) is located at the button of the parameter window. The change to cuvette/nanovolume button toggles between nanovolume and cuvette applications. Selecting the Change to Cuvette button activates the cuvette compartment (opens the sliding cover and switches the red arrow LED on). Only cuvette measurements are possible when this mode is selected. Selecting the Change to Cuvette button while it is activated will deactivates the cuvette compartment (closes the sliding cover and switches the LED off) and only nanovolume measurements are possible.

Note: Please make sure that the nanovolume arm is closed during cuvette measurements.

The Change to NanoVolume button (C40) is only shown when submicroliter cell applications are enabled. The Change to NanoVolume/Cuvette button toggles between cuvette and submicroliter cell applications. With the NanoVolume submicroliter application the parameter of each method show a dropdown for lid selection (Lid 5, 10, 50, 100 and 250). Choose the correct lid and the results are automatically calculated considering the virtual dilution.

SIDE TAB BAR

On the left side of the measurement screen there is a vertical tab bar that contains four tabs including: parameters, data, graph, and table. The different tabs allow organizing the measurement screen. It is possible to show or hide the different areas on the screen. Default screen for computer show all areas, for the built-in screen and tablet version the table is hidden.



Note: There is no tab bar available for smart phone versions. The parameters, results and graph screens are shown full screen. Parameters need to be confirmed (>) to get to the measurement screen. It is possible to toggle between the results and graph area by sliding left and right. There is no table area available on smart phones.

- **Parameter area**

In the parameter area it is possible to define all necessary parameters for a measurement as well as turn on cuvette mode and initiate cuvette heating. The default measurement screen includes the parameter area as open. It is automatically hidden when starting either a blank or sample measurement by clicking on the Blank or Sample button. It is also possible to hide the parameter area by tapping the parameter tab in the vertical side tab bar.

- **Results area**

The results area shows the method specific results of the latest measurement including concentration, absorbances, and relevant ratios from the last sample measured. It is also possible to change the units of the calculated concentrations in the results area with a drop-down selection menu.

Note: Selection of one or more than one line is selected in the table no data are shown in the result view.

- **Table area**

The table area collects defined results for all samples completed during the active method. The first column of the table indicates whether the measurement is saved (💾) or not saved (blank field).

- **Graph area**

The graph area shows a chart with the graph of the actual measurement or the selected line(s) in the table. There is an overlay toggle switch on the left bottom of the graph area. If the overlay option is enabled the graphs of the measurements will be automatically overlaid. To change the overlaid graphs select/deselect the samples in the table.

Note: It is only possible to overlay up to 30 graphs in a chart. If more than 30 data are selected a message will appear that says “Too many data selected. Up to 30 data can be overlaid.” Please select the desired data to be displayed on the graph.

Note: The overlay button is not available on the NanoPhotometer and smart phones, only on tablets and computer versions.

It is possible to zoom in and out any area of the diagram (x- and y-axis). Undo the zoom by clicking the full scale icon (⤡).

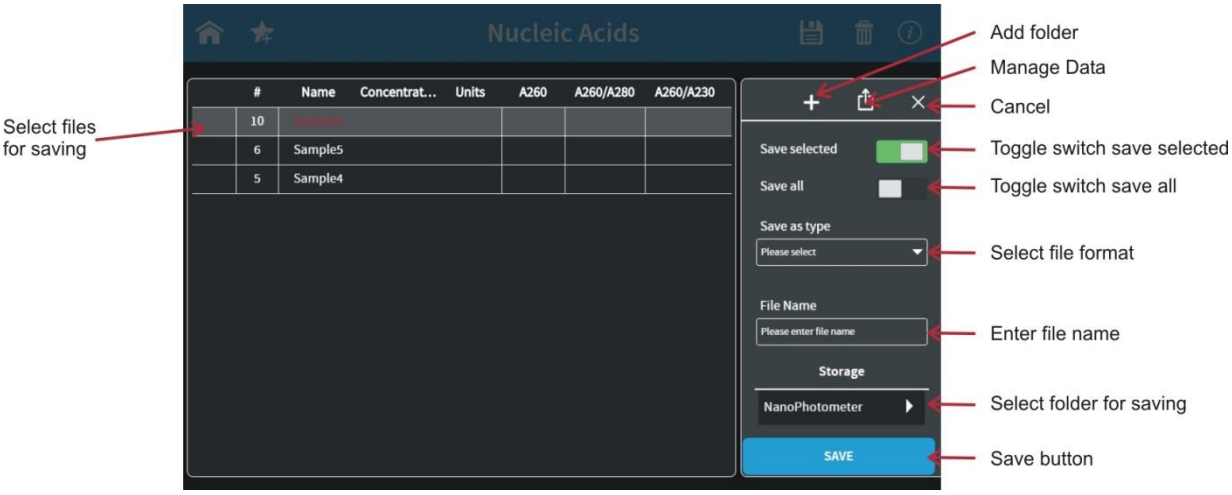
Note: Maximum zoom is 20 nm for the x-axis and 0.01A for the y-axis.

As legend option the sample name in the table is colored in the same color as the graph in the chart.
Clicking on the graph opens a pop up which shows the sample name, wavelength and absorbance of the selected wavelength. It is possible to show results of further graphs by changing the sample in the sample name drop-down.

DATA PROCESSING DIALOGS

- **Save**

Selecting the save data icon (💾) opens a full screen overlay dialog window with various save options.



Save selected

This option saves the current selection of data of the table. If the save selected toggle switch is green the save all toggle switch will automatically be deactivated.

Save all

The save all button (default setting) allows the user to save all of the data from the current method. If the save all toggle switch is green the save selected toggle switch will automatically be deactivated.

Note: On the smart phone app there is no save selected/save all toggle switch available; instead in this case the entire measurement session is saved.

Save as

The save as button allows the user to specify which type of file to save the data as. The options of file types include: Excel and Implen Document Source (IDS), which can be saved to NanoPhotometer, Control Device and on USB flash drive.

Note: Control Device is only available on smart phones and tablets.

Implen Document Source File

Implen has a custom file format referred to as being an Implen Document Source (IDS) file. This is a hardcopy file to which no changes can be made. This file type contains all information including raw data, results, values, and parameters. The saved file shows only the selected information at the time the file was saved. IDS files can only be opened with the Implen software. The data are opened in the measurement.

Excel File

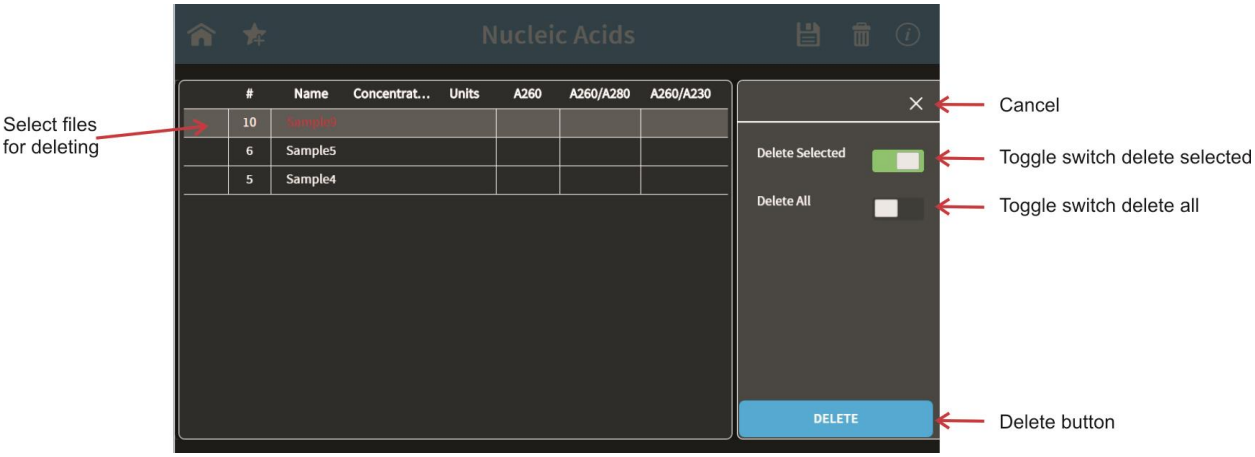
Data from the measurements can also be saved in an Excel file format. This file format is compatible with Microsoft Excel software versions 2007/2010/2013 and can be edited. This file type contains all information including raw data, results, and parameters. The saved file contains only the selected information at the time the file was saved.

Storage

Selection option to choose the device the data should be saved to. The options include: NanoPhotometer, USB flash drive (if connected) and Control Device (for smart phones and tablets only). If Control Device is selected the data will be transferred to the device that is currently connected with the NanoPhotometer.

- **Delete**

Selecting the delete icon (🗑) opens a full screen overlay dialog window with delete options.



Delete selected

This option deletes the current selection of data in the table. If the delete selected toggle switch is green the delete all toggle switch will automatically be deactivated.

Delete all

The delete all button allows the user to delete all of the data from the current method. If the save all toggle switch is green the save selected toggle switch will automatically be deactivated.

Once the delete button is selected a message will appear asking “Do you want to delete all/selected files?” selecting cancel will return to the delete menu screen and selecting delete will delete the selected data.

Note: The delete function is not available for the software version designed for smart phones.

BASIC OPERATION

The NanoPhotometer product line offers a complete solution for nanovolume (N50/N60/NP80/C40) and standard volume (C40/NP80) applications. With the NanoPhotometer nanovolume applications the sample volume ranges from 0.3 µl to a maximum sample volume of 2.0 µl. Standard Cuvette applications can be performed with 10 mm, 5 mm, 2 mm and 1 mm path length quartz, glass, or plastic cuvettes with a center height of 8.5 cm.

Note: NanoVolume application in the NanoPhotometer C40 is only available with the optional accessory “submicroliter cell” from Implen.

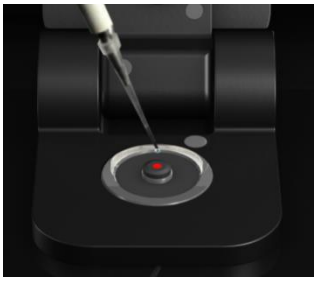
NANOVOLUME MEASUREMENT BASICS (N50/N60/NP80)

1. Select a method depending on your sample and set the parameter for the measurement.

2. Ensure that the sample window and the mirror in the sample arm are clean.

3. Use the integrated vortexer (N60 and NP80 only) to mix your sample to achieve an homogenous sample

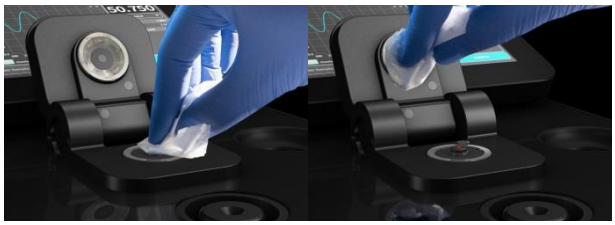
4. Raise the sample arm and pipette the appropriate amount of sample onto the illuminated sample window. The illumination turns automatically off, lowering the arm.



Note: Do not overfill the well.
Note: Save, low energy red light (LED)

5. Lower the sample arm and initiate a blank measurement with the blank button

6. Clean the measurement window and mirror on the sample arm with a slightly wet lint-free tissue. Use water, 70% ethanol or isopropanol.



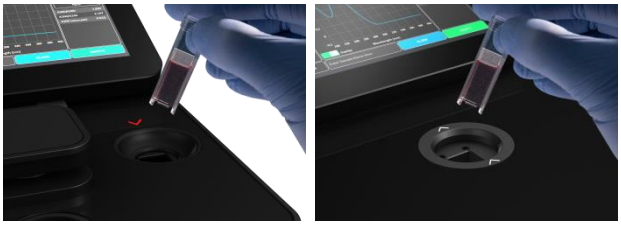
Note: Make sure that the metal contact face (around the measurement window and the mirror) is clean.
Note: Do not use aggressive solvents such as strong acids or bases or organic solvents at any time. If unsure please contact support@implen.de for detailed information about your specific reagent/solvent.

7. Raise the sample arm and pipette the appropriate amount of sample solution onto the illuminated sample window (1 - 2 µl for automatic path length change or 0.3 µl for dsDNA > 120 ng/µl / BSA > 12.6 mg/ml). Upon completion of measurement raise sample arm, clean the surfaces and apply the next sample.

Note: The sample window must be clean and residual fluff from any cleaning wipe must be removed for optimum performance.

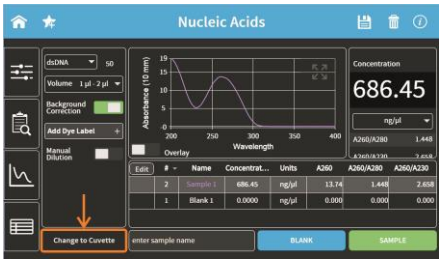
CUVETTE MEASUREMENT BASICS (C40/NP80)

The NanoPhotometer (NP80 and C40 only) is compatible with standard cuvettes having an 8.5 mm center height. The light path is indicated with a red status LED arrow for the NP80 model and with two white arrows for the C40 model.



Note: For the NP80 the cuvette compartment needs to be activated by the “Change to Cuvette” button in the parameter area. Once the cuvette option is activated the sample compartment door will be opened automatically and a red arrow above the cuvette compartment will appear. The arrow indicates the light path.

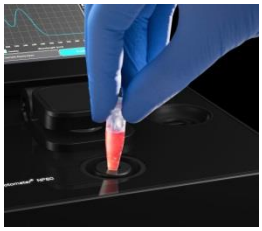
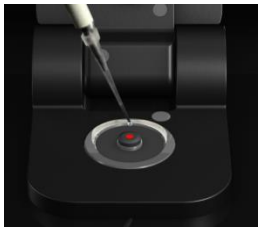
1. Open the cuvette cover by clicking the “Change to Cuvette” button below the parameter area (NP80).



2. Add a blank solution to the cuvette and ensure that the filling volume is sufficient to allow light path to pass through the solution.
3. Insert cuvette into the cell holder.
4. Initiate a blank measurement with the blank button. Upon completion of measurement remove the cuvette.
5. Add sample to cuvette and ensure that the sample volume is sufficient to allow light to pass through the sample.
6. Initiate a sample measurement with the sample button. Upon completion of measurement remove the cuvette
7. Apply further samples.

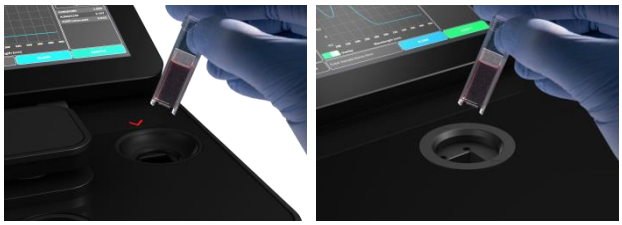
SAMPLE HANDLING TIPS

- **Nanovolume Methods**

- The NanoPhotometer includes an integrated vortexer (N60/NP80 only) to assure sample homogeneity. It is recommended to vortex every sample right before the measurement.
- The sample window is illuminated with a low energy red light to assist with accurate sample application. The red light is switched off once the sample arm is closed. It is possible to disable the illumination feature in preferences of the NPOS.
- The minimum volume that can be used for nanovolume samples is 0.3 µl (starting for dsDNA > 120 ng/µl and BSA > 12.6 mg/ml). For automatic path length setting at least 1µl is needed.
- The maximum volume that can be used for nanovolume samples is 2.0 µl.
- The sample can be fully recovered after measurement with a pipette if desired.
  Note: Minimal cross contamination cannot be avoided on a molecular level.
- Proper cleaning is important to ensure accurate measurements. In most cases a dry lint-free laboratory wipe is sufficient to clean the sample quartz surfaces. In the case of highly concentrated samples or certain proteins, the recommended procedure for cleaning is to use a slightly wet lint-free laboratory wipe (with water or 70% EtOH depending on sample type) to thoroughly clean the sample surface.
- It is mandatory that the metal contact face around the measurement window and the mirror is clean.

- **Cuvette Methods**



- The cuvette holder (NP80/C40 only) is compatible with standard 10 mm path length quartz, glass and plastic cuvettes with an optical height of 8.5 mm.
- It is also possible to use cuvettes with 5 mm, 2 mm or 1 mm path lengths, but there may be an adapter necessary. Please ask your cuvette supplier for suitable adapter.
- The minimum volume for accurate measurements depends on the cuvette type used; it is necessary for the light path to pass through the sample for accurate measurements.

Note: The cuvette holder is not removable. Do not pour any cleaning solution into the cuvette holder as larger amounts of liquids can get into the instrument and cause damage.

Solvent Compatibility (N50/N60/NP80)

Most solvents typically used in life science laboratories are compatible with the NanoPhotometer nanovolume sample surfaces. The following solvents are compatible for use with the NanoPhotometer models N50, N60, and NP80 at room temperature:

- Acetone (≤ 5%)
- Acetonitrile
- Benzene
- Butanol
- Carbon tetrachloride
- Chloroform
- Ether
- HEPES
- Hexane
- Isopropanol
- MES
- Methanol
- Methylenchlorid
- MOPS
- Phenol (≤ 1%)
- N-propanol
- Toluene
- Phosphate containing buffers
- PBS (pH 4-10)
- Citrate
- Borate
- Chloride salts
- Acids > pH 2
- Bases < pH 10

Note: Highly concentrated acids and bases are not recommended. It is recommended to wipe the sample surface with a lint-free laboratory wipe immediately upon completion of each measurement. For more information about compatibility of specific solvents not listed above, please contact the Implen support team (support@implen.de) to check the compatibility.

BATTERY OPERATION

The optional battery operation is only available for NP80-Touch, N60-Touch and C40-Touch models. A full battery pack can be operated for approximately 8 hours within normal usage, i.e.

20-30 measurements per hour. When the battery gets low there will be a first warning message “Battery low” and a bleep is sounded. At this status it is possible to use the instrument under standard conditions at least for 1 hour. It is recommended to charge the battery at this status. If the battery is not charged there will be a second warning message when the battery is empty and several beep sounds before the instrument is automatically shutting down within 10 to 15 seconds.

Note: Unsaved data can be found in the autosave folder in Stored Methods. If the NanoPhotometer is shutting down during a measurement process the data of this measurement are lost.

The battery pack is designed for 800 full charging cycles. Afterwards the capacity and operation time of the battery may vary. A fully charged battery pack will self-discharge (switched off NanoPhotometer) over a time of 10 – 14 days. After this time recharging of the battery pack is necessary.

4. NanoPhotometer Applications

The NanoPhotometer comes with pre-programmed applications as well as the ability to create custom applications. Any application method can be selected by tapping the icon once or clicking on the icon (computer based software).

Note: To select a method select the corresponding icon. Selection of nanovolume or cuvette mode is within each method possible. The “Change to Cuvette” or “Change to NanoVolume” button is located at the bottom of the parameter area.

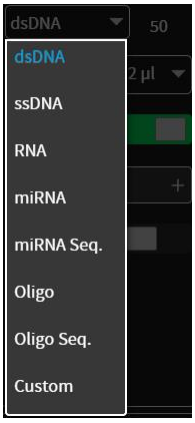
Nucleic Acids

Method Overview

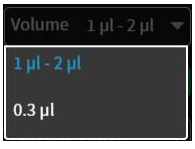
Nucleic acids in solution absorb light with a peak in the ultraviolet region of 260 nm. For determination of nucleic acid concentration in solution the absorbance at wavelength 260 nm is used along with the Beer-Lambert law. In addition to calculating concentrations of nucleic acids, absorbance measurements are also useful for estimating purity of nucleic acids by calculating the 260:280 nm and 260:230 nm ratios. Further, it is possible to determine the degree of labeling of nucleic acids with probes including fluorescent dyes.

Measurements Protocol

1. Select the Nucleic Acids icon on the home screen.
   To change between NanoVolume and cuvette application (NP80/C40 only) use the Change to Cuvette/NanoVolume button below the parameter area.

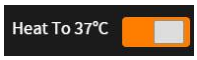


2. Select the nucleic acid type in the drop-down of the parameter area.
   Options are: dsDNA, ssDNA, RNA miRNA, miRNA Sequence, Oligo, Oligo Sequence and Custom (see Table 1).
   - The miRNA sequence and Oligo sequence options allow having the sequence entered and the extinction coefficient will be automatically calculated.
   - Custom is set to 50 as a default and allows the extinction coefficient to be manually entered from the range of 15 - 150 up to three decimal points.



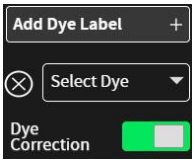
3. **For NanoVolume application:**
   Select the volume of sample to be applied.
   Note: 1 - 2 µl (default): automatic path length change; 0.3 µl measures only the 0.07 mm path length (for samples with concentrations >120 ng/µl dsDNA)

For cuvette application:
Select the path length depending on the used cuvette.
Options are: 1 mm, 2 mm, 5 mm and 10 mm



4. If it is desired to heat the sample to 37°C use the toggle switch to turn on cell holder heater. When the cuvette holder has 37°C the color changes to green.
Note: Only available for cuvette applications (NP80 and C40).

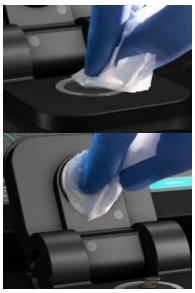
5. The background correction is enabled as the default. It is possible to disable the background correction with the toggle switch.



6. If a dye labeled sample is used please add a dye by tapping on the Add Dye Label option and select a dye from the drop-down list. There is also an option for dye correction which can be en-/disabled with a toggle switch. Added Dyes can be deleted by tapping on the delete icon (⊗).
Note: If the used dye is not available in the drop-down list, please go to preferences and add a custom dye to the dye list.

7. Option to set/calculate a dilution factor for manual diluted samples.

8. Apply the blank ddH20 or buffer to the illuminated sample window for the reference measurement and select blank to initiate the reading.
Note: Use always a minimum of 1 µl for any blank measurement.
Note: The illumination of the sample window can be switched off in the preferences.



9. Use a lint-free laboratory wipe to clean both the sample window and mirror prior to applying the next sample.
Note: It could be helpful to apply the blank a second time and read it as a sample to ensure a proper blank.

10. Apply sample to the sample window and press the sample button to initiate the measurement.

CALCULATIONS

- **Nucleic Acid Concentration**

To determine the concentration of nucleic acids in solution, the absorbance as measured at the wavelength of 260 nm is used. The function describing the relationship between concentration and absorbance is a modification of the Beer-Lambert law equation. The concentration of the

nucleic acid samples can be calculated with or without background correction depending on if the background correction is turned on for the measurements.

Without background correction:

$C = A_{260} * \varepsilon_{nuc} * Đ$

With background correction:

$C = (A_{260} - A_{320}) * \varepsilon_{nuc} * Đ$

C	Concentration in ng/µl
A_{260}	Absorbance at 260 nm (10 mm path)
A_{320}	Absorbance at 320 nm (10 mm path)
Đ	Manual dilution factor
ε_{nuc}	Extinction coefficient/nucleic acid factor in ng*cm/µl

Table 1. Nucleic acids extinction coefficients (ε_{nuc})

Type	ε_{nuc}
dsDNA	50 ng*cm/µl
ssDNA	37 ng*cm/µl
RNA	40 ng*cm/µl
miRNA	33 ng*cm/µl
Oligo	33 ng*cm/µl
miRNA Seq.	calculated via extinction coefficient of constituent nucleotides entered
Oligo Seq.	calculated via extinction coefficient of constituent nucleotides entered
Custom	Option to enter any factor between 15 and 150 ng*cm/µl

- **Dye-labeled Nucleic Acid Concentration**

For dye-labeled nucleic acids, the concentration of the nucleic acid is calculated using a modified form of the Beer-Lambert equation. For these calculations, the instrument considers the absorption maximum of the dye and a certain dye-specific correction factor at 260 nm (see Table 2). The concentration of a dye-labeled nucleic acid is calculated with or without background/dye correction as follows:

With background and with dye correction:

$$C = \left[(A_{260} - A_{320}) - \left(cf_{dye} * (A_{max,dye} - A_{320})\right)\right] * \varepsilon_{nuc} * Đ$$

With background and without dye correction:

$C = (A_{260} - A_{320}) * \varepsilon_{nuc} * Đ$

Without background and with dye correction:

$$C = [A_{260} - cf_{dye} * A_{max,dye}] * \varepsilon_{nuc} * Đ$$

Without background and without dye correction:

$$C = A_{260} * \varepsilon_{nuc} * Đ$$

C	Concentration in ng/µl
A_{260}	Absorbance at 260 nm (10 mm path)
A_{320}	Absorbance at 320 nm (10 mm path)
$A_{max,dye}$	Absorbance value at the absorbance maximum of the dye (10 mm path)
ε_{nuc}	Extinction coefficient/nucleic acid factor in ng*cm/µl
cf_{dye}	Dye-dependent correction factor at 260 nm
Đ	Manual dilution factor

- **Dye Concentration**

For dye-labeled nucleic acids, the concentration of the dye is calculated using a modified form of the Beer-Lambert equation. For these calculations, the instrument considers the absorption maximum of the dye, the dye-specific extinction coefficient (see Table 2). The dye concentration is calculated with or without background correction as follows:

With background correction:

$$C = \frac{(A_{max,dye} - A_{320}) * Đ}{\varepsilon_{dye} * 10^6}$$

Without background correction:

$$C = \frac{A_{max,dye} * Đ}{\varepsilon_{dye} * 10^6}$$

C	Concentration in ng/µl
$A_{max,dye}$	Absorbance value at the absorbance maximum of the dye (10 mm path)
A_{320}	Absorbance at 320 nm (10 mm path)
ε_{dye}	Extinction coefficient of dye in $M^{-1}*cm^{-1}$
Đ	Manual dilution factor

- **Frequency of Incorporation (FOI)**

FOI is the degree of labeling based on dye incorporation in a labeled nucleic acid sample. It is generally expressed as the number of dye molecules incorporated per 1000 nucleotides. FOI can be calculated with or without background/dye correction as follows:

With background correction with dye correction:

$$FOI = \frac{324.5 * (A_{max,\,dye} - A_{320})}{\varepsilon_{dye} * 10^6 * \left(A_{260} - A_{320} - cf_{dye} * (A_{max,dye} - A_{320})\right)}$$

With background correction without dye correction:

$$FOI = \frac{324.5 * (A_{max,\,dye} - A_{320})}{\varepsilon_{dye} * 10^6 * (A_{260} - A_{320}) * \varepsilon_{nuc}}$$

Without background correction and with dye correction:

$$FOI = \frac{324.5 * A_{max,\,dye}}{\varepsilon_{dye} * 10^6 * (A_{260} - cf_{dye} * A_{max,dye}) * \varepsilon_{nuc}}$$

Without background correction and without dye correction:

$$FOI = \frac{324.5 * A_{max,\,dye}}{\varepsilon_{dye} * 10^6 * A_{260} * \varepsilon_{nuc}}$$

FOI	Frequency of Incorporation (dye per 1,000 bases)
A_{260}	Absorbance at 260 nm (10 mm path)
A_{320}	Absorbance at 320 nm (10 mm path)
$A_{max,dye}$	Absorbance value at the absorbance maximum of the dye (10 mm path)
ε_{dye}	Extinction coefficient of dye in $M^{-1}*cm^{-1}$
ε_{nuc}	Extinction coefficient/nucleic acid factor in ng*cm/µl
cf_{dye}	Dye-dependent correction factor at 260 nm

Table 2. Dye Types, Absorbance Max, Extinction coefficient, and dye-dependent correction factors

NanoPhotometer Model	Dye Type	Absorbance maximum of Dye (nm)	Dye-dependent ext. coeff. ε_{dye} in $M^{-1}*cm^{-1}$	Dye-dependent correction factor at 260 nm cf_{dye}
NP80/N60/N50/C40	Alexa Fluor 350	345	18,400	0.25
NP80/N60/N50/C40	Alexa Fluor 488	492	62,000	0.30
NP80/N60/N50/C40	Alexa Fluor 532	525	82,300	0.24
NP80/N60/N50/C40	Alexa Fluor 546	555	104,000	0.21
NP80/N60/N50/C40	Alexa Fluor 555	555	150,000	0.04
NP80/N60/N50/C40	Alexa Fluor 568	576	93,000	0.45
NP80/N60/N50/C40	Alexa Fluor 594	588	80,400	0.43
NP80/N60/C40	Alexa Fluor 647	650	239,000	0.00

NP80/N60/C40	Alexa Fluor 660	660	107,000	0.00
NP80/N60/C40	Alexa Fluor 680	680	164,000	0.00
NP80/N60/N50/C40	Cy3	550	150,000	0.08
NP80/N60/N50/C40	Cy3.5	581	150,000	0.08
NP80/N60/C40	Cy5	649	250,000	0.05
NP80/N60/C40	Cy5.5	675	250,000	0.05
NP80/N60/N50/C40	Oyster-500	503	78,000	0.29
NP80/N60/N50/C40	Oyster-550	553	150,000	0.05
NP80/N60/N50/C40	Oyster-556	560	155,000	0.03
NP80/N60/C40	Oyster-645	649	220,000	0.05
NP80/N60/C40	Oyster-650	653	200,000	0.04
NP80/N60/C40	Oyster-656	660	200,000	0.04
NP80/N60/N50/C40	Texas Red	593	85,000	0.23

- **Ratios**

Reactions utilizing nucleic acids often require minimum purity standards. Common contaminants of nucleic acid samples include: proteins, organic compounds, and other. Based on the common contaminants of nucleic acid samples, the 260/280 and 260/230 ratios are calculated for nucleic acids to give an indication of the purity of the samples. Pure DNA and RNA preparations have expected 260/280 ratios of ≥ 1.8 and ≥ 2.0 respectively. An elevated absorbance at 230 nm can indicate the presence of impurities as well; 230 nm is near the absorbance maximum of peptide bonds and also indicates buffer contamination since TRIS, EDTA and other buffer salts absorb at 230nm. When measuring RNA samples, the 260/230 ratio should be > 2.0; a ratio lower than this is generally indicative of contamination with guanidinium thiocyanate, a reagent commonly used in RNA purification and which absorbs over the 230 - 260 nm range.
The ratios are calculated with or without background correction according to if the background correction is activated during the measurements or not as follows:

Without background correction:

$$260/280 \text{ ratio} = \frac{A_{260}}{A_{280}}$$

$$260/230 \text{ ratio} = \frac{A_{260}}{A_{230}}$$

With background correction:

$$260/280 \text{ ratio} = \frac{A_{260} - A_{320}}{A_{280} - A_{320}}$$

$$260/230 \text{ ratio} = \frac{A_{260} - A_{320}}{A_{230} - A_{320}}$$

PROTEIN UV

METHOD OVERVIEW

The Protein UV method exploits the inherent absorbance of proteins at 280 nm in combination with the Beer-Lambert Law, where each protein is characterized by a protein specific extinction coefficient (ε) which can be used to determine total protein concentration of a solution. The intrinsic absorbance of proteins is due to the presence of aromatic amino acids in their structure, primarily tryptophan and tyrosine, as well as cysteine (oxidized cysteine residues in a disulphide bond). The aromatic amino acid residues in a protein containing tryptophan and tyrosine exhibit strong intrinsic absorbance at 280 nm, with a lesser contribution by phenylalanine. Therefore, it is the aromatic amino acid residues which dictate the extinction coefficient at 280 nm for a protein.

The most straightforward method to determine concentration of a purified, homogenous protein with a known extinction coefficient (ε) is by direct measurement of UV280 provided as long as the protein contains no prosthetic groups with strong absorption in the same region. However, for unknown proteins including homogenous protein mixtures, it is possible to make direct A_{280} measurements using a composite ε value derived from comparison of many proteins, although this will only provide an approximate but close estimate of the true protein concentration.

The NanoPhotometer determines protein concentration by performing calculations based on specific ε values, either pre-programmed in the instrument or entered manually by the user. Extinction coefficient (ε) values at 280 nm vary greatly for different proteins due to their particular aromatic amino acid content. Fixed ε values are pre-programmed in the software for certain proteins (see

Table 3). However, if the protein of interest is not included in the pre-programed methods it is also possible to manually enter the specific ε for the protein of interest using the Mol. Ext. Coefficient or Ext. Coefficient protein option. For correct calculation, it is necessary to supply either: the mass extinction coefficient (ε in l/g*cm); or b) or the molar extinction coefficient (ε_M in $M^{-1}*cm^{-1}$), and the molecular weight of the protein expressed in molar mass units (g/mol).

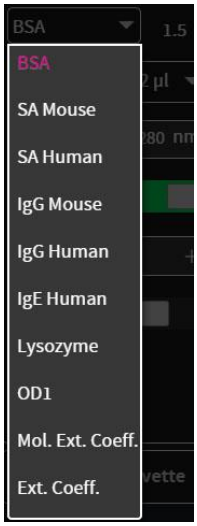
To determine the degree of dye labelling of a protein, the absorbance measured at the wavelength corresponding to the absorbance maximum of the fluorescence dye is used (see

Table 3). The corresponding extinction coefficient of the dye is used along with the in the Beer-Lambert Law to determine the dye concentration.

Note: It is important to ensure the extinction coefficient and units entered are correct in order to ensure that calculations are performed properly for accurate concentration values.

MEASUREMENTS PROTOCOL

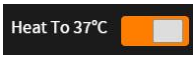
1. Select the Protein UV icon on the home screen.
   To change between NanoVolume and cuvette application (NP80/C40 only) use the Change to Cuvette/NanoVolume button below the parameter area.



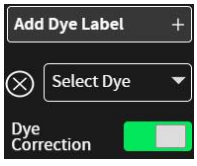
2. Select the protein type in the drop-down of the parameter area:
   Options are: BSA, SA Mouse, SA Human, IgG Mouse, IgG Human, IgE Human, Lysozyme, OD1, Mol. Ext. Coefficient, Ext. Coefficient.
   - For Mol. Ext. Coefficient enter Molecular Weight (default 66,400 g/mol) and Mol. Ext. Coefficient (default 44,289 $M^{-1}*cm^{-1}$)
   - For Ext. Coefficient enter Ext. Coefficient (default 0.667 l/g*cm)

3. **For NanoVolume application:**
   Select the volume of sample to be applied.
   Note: 1 - 2 µl (default): automatic path length change; 0.3 µl measures only the 0.07 mm path length (possible for samples with concentrations e.g. BSA > 12.6 mg/ml)
   For cuvette application:
   Select the path length depending on the used cuvette.
   Options are: 1 mm, 2 mm, 5 mm and 10 mm

4. The background correction is enabled as the default. It is possible to disable the background correction with the toggle switch.



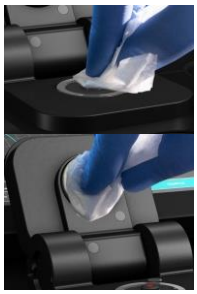
5. If it is desired to heat the sample to 37°C use the toggle switch to turn on cell holder heater. When the cuvette holder has 37°C the color changes to green.
   Note: Only available for cuvette applications (NP80 and C40).



6. If a dye labeled sample is used please add a dye by tapping on the Add Dye Label option and select a dye from the drop-down list. There is also an option for dye correction which can be en-/disabled with a toggle switch. Added Dyes can be deleted by tapping on the delete icon (⊗).
   Note: If the used dye is not available in the drop-down list, please go to preferences and add a custom dye to the dye list.

7. Option to set/calculate a dilution factor for manual diluted samples.

8. Apply the blank ddH20 or buffer to the illuminated sample window for the reference measurement and select blank to initiate the reading.
   Note: Use always a minimum of 1 µl for any blank measurement.
   Note: The illumination of the sample window can be switched off in the preferences.



9. Use a lint-free laboratory wipe to clean both the sample window and mirror prior to applying the next sample.
   Note: It could be helpful to apply the blank a second time and read it as a sample to ensure a proper blank.

10. Apply sample to the sample window and press the sample button to initiate the measurement.

CALCULATIONS

- **Protein UV280 Concentration**

The protein concentration in the Protein UV method is calculated with the absorbance value of the sample at 280 nm along with the extinction coefficient defined by the user. The protein concentration is calculated with or without background correction as follows:

With background correction:

$C = (A_{280} - A_{320}) * \varepsilon_{prot} * Đ$

Without background correction:

$C = A_{280} * \varepsilon_{prot} * Đ$

C	Concentration (ng/µl)
A_{280}	Absorbance at 280 nm (10 mm path)
A_{320}	Absorbance at 320 nm (10 mm path)
ε_{prot}	Extinction coefficient/protein factor in g*cm/l
Đ	Dilution factor

Table 3. Protein extinction coefficients (ε_{prot})

Type	ε_{prot} [g* cm/l]	Ext. Coeff. [l/g*cm]	Mol. Ext. Coeff. [M^{-1}*cm^{-1}]	MW [g/mol]
BSA	1.50	0.667	44,289	66,400
SA Mouse	1.49	0.670	44,220	66,000
SA Human	1.72	0.582	40,370	69,365
IgG Mouse	0.71	1.400	224,000	160,000
IgG Human	0.74	1.360	204,000	150,000
IgE Human	0.65	1.530	290,700	190,000
Lysozyme	0.38	2.640	37,984	37,984
OD1	1.00	N/A	N/A	N/A

- **Dye-labeled Protein UV280 Concentration**

For dye-labeled proteins, the concentration of the protein is calculated using a modified form of the Beer-Lambert equation. For these calculations, the instrument considers the absorption maximum of the dye, and a certain dye-specific correction factor at 280 nm (see Table 4). The dye concentration is calculated with or without background/dye correction for are as follows:

With background and with dye correction:

$$C = [A_{280} - A_{320} - cf_{dye} * (A_{max,dye} - A_{320})] * \varepsilon_{prot} * Đ$$

With background and without dye correction:

$$C = (A_{280} - A_{320}) * \varepsilon_{prot} * Đ$$

Without background and with dye correction:

$$C = (A_{280} - cf_{dye} * A_{max,dye}) * \varepsilon_{prot} * Đ$$

Without background and without dye correction:

$$C = A_{280} * \varepsilon_{prot} * Đ$$

C	Concentration in mg/ml
A_{280}	Absorbance at 280 nm (10 mm path)
A_{320}	Absorbance at 320 nm (10 mm path)
$A_{max,dye}$	Absorbance value at the absorbance maximum of the dye (10 mm path)
ε_{prot}	Extinction coefficient/protein factor in g*cm/l
cf_{dye}	Dye-dependent correction factor at 280 nm
Đ	Dilution factor

- **Dye Concentration**

For dye-labeled proteins, the concentration of the dye is calculated using a modified form of the Beer-Lambert equation. For these calculations, the instrument considers the absorption maximum of the dye, and a dye-specific extinction coefficient (see Table 4). The dye concentration is calculated with or without background correction for are as follows:

With background correction:

$$C = \frac{\left((A_{max,dye} - A_{320}) * Đ\right)}{\varepsilon_{dye} * 10^6}$$

Without background correction:

$$C = \frac{(A_{max,dye} * Đ)}{\varepsilon_{dye} * 10^6}$$

C	Concentration
$A_{max,\ dye}$	Absorbance at the max dye absorbance value (10 mm path)
A_{320}	Absorbance at 320 nm (10 mm path)
ε_{dye}	Extinction coefficient of dye in $M^{-1}*cm^{-1}$
Đ	Dilution factor

- **Degree of Labeling (DOL)**

DOL is the degree of labeling based on the average number of dye molecules coupled to a protein molecule. The degree of labeling can be determined from the absorption spectrum of the labeled antibody with or without background/dye correction as follows:

With background and with dye correction:

$$DOL = \frac{(A_{max,\ dye} - A_{320}) * \varepsilon_{prot}}{\left(A_{280} - A_{320} - cf_{dye} * (A_{max,\ dye} - A_{320})\right) * \varepsilon_{dye}}$$

With background and w/o dye correction:

$$DOL = \frac{(A_{max,\ dye} - A_{320}) * \varepsilon_{prot}}{(A_{280} - A_{320}) * \varepsilon_{dye}}$$

W/o background and with dye correction:

$$DOL = \frac{A_{max,\ dye} * \varepsilon_{prot}}{(A_{280} - cf_{dye} * A_{max,\ dye}) * \varepsilon_{dye}}$$

W/o background and w/o dye correction:

$$DOL = \frac{A_{max,\ dye} * \varepsilon_{prot}}{A_{280} * \varepsilon_{dye}}$$

DOL	Degree of labeling/dye per protein ratio
$A_{max,dye}$	Absorbance value at the absorbance maximum of the dye (10 mm path)
A_{280}	Absorbance at 280 nm (10 mm path)
A_{320}	Absorbance at 320 nm (10 mm path)
ε_{dye}	Extinction coefficient of dye in $M^{-1}*cm^{-1}$
ε_{prot}	Extinction coefficient/protein factor in g*cm/l
cf_{dye}	Dye-dependent correction factor at 280 nm

Table 4. Dye Types, Absorbance Max, Extinction coefficients, and dye-dependent correction factors

NanoPhotometer Models	Dye Type	Absorbance maximum of Dye (nm)	Dye-dependent ext. coeff. ε_{dye} in $M^{-1}*cm^{-1}$	Dye-dependent correction factor at 280 nm cf_{dye}
NP80/N60/N50/C40	Alexa Fluor 350	346	19,000	0.19
NP80/N60/N50/C40	Alexa Fluor 405	401	34,500	0.70
NP80/N60/N50/C40	Alexa Fluor 488	494	71,000	0.11
NP80/N60/N50/C40	Alexa Fluor 532	530	81,000	0.09
NP80/N60/N50/C40	Alexa Fluor 546	554	104,000	0.12
NP80/N60/N50/C40	Alexa Fluor 555	555	150,000	0.08
NP80/N60/N50/C40	Alexa Fluor 568	577	91,300	0.46
NP80/N60/N50/C40	Alexa Fluor 594	590	73,000	0.56
NP80/N60/C40	Alexa Fluor 647	650	239,000	0.03
NP80/N60/C40	Alexa Fluor 680	679	184,000	0.05
NP80/N60/C40	Alexa Fluor 790	785	260,000	0.08
NP80/N60/N50/C40	Cy3	550	150,000	0.05
NP80/N60/C40	Cy5	649	250,000	0.05
NP80/N60/C40	DyLight 649	654	250,000	0.04
NP80/N60/N50/C40	DyLight 488	493	70,000	0.15
NP80/N60/N50/C40	FITC	495	68,000	0.30
NP80/N60/N50/C40	Pacific Blue	409	30,000	0.20
NP80/N60/N50/C40	Pacific Orange	397	24,500	0.60
NP80/N60/N50/C40	pHrodo Green	505	75,000	0.20
NP80/N60/N50/C40	pHrodo Red	560	65,000	0.12
NP80/N60/N50/C40	r-PE	566	200,000	0.18
NP80/N60/N50/C40	Texas Red	595	80,000	0.18

PROTEIN ASSAYS

METHOD OVERVIEW

Protein concentration may be measured using colorimetric assays, in which certain reagents are added to the protein solution to generate a colored product; either a protein-cupric ion chelate as in the Biuret, Lowry, BCA assays or a protein-dye complex as in the Bradford assay. In these colorimetric assays, the absorbance is measured in the visible range at the appropriate wavelength for each assay and compared against a standard curve prepared by serial dilution of a protein standard of known concentration. A linear regression analysis of the calibration standard data points is calculated by the NanoPhotometer. A correlation coefficient (R^2) in the range of 0.95 to 1.00 indicates a good fit to a straight line.

- **Bradford Assay**

Method depends on quantifying the binding of a dye, Coomassie Brilliant Blue, to an unknown protein and comparing this binding to that of a standard curve prepared from a set of known protein of known concentrations at 595 nm. This standard is usually BSA (bovine serum albumin).

- **Biuret Assay**

Method depends on a reaction between cupric ions and peptide bonds in an alkali solution, resulting in the formation of a complex absorbing at 546 nm.

- **BCA Assay**

Method depends on a reaction between cupric ions and peptide bonds coupled with the detection of cuprous ions using bicinchoninic acid (BCA), giving an absorbance maximum at 562 nm. The BCA process is less sensitive to the presence of detergents used to solubilize membranes.

- **Lowry Assay**

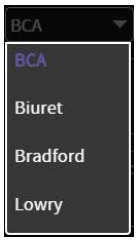
Method is based on the Biuret reaction. Under alkaline conditions the divalent copper ion forms a complex with peptide bonds in which it is reduced to a monovalent ion. Monovalent copper ion and the radical groups of tyrosine, tryptophan, and cysteine react with Folin reagent to produce an unstable product that becomes reduced to molybdenum/tungsten blue. The bound reagent changes color from yellow to blue. This binding is compared with that obtained with a standard protein at 750 nm; this is usually BSA (bovine serum albumin).

Note: Detailed protocols are customarily supplied with these assay kits, and must be closely followed to ensure that accurate results are obtained.

MEASUREMENTS PROTOCOL

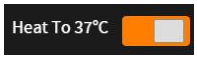
Note: It is not recommended to use the NanoVolume application for Protein Assays. For best results use the cuvette mode (NP80 and C40 models only) for the Protein Assays.

1. Select protein assays icon from home screen.
   To change between NanoVolume and cuvette application, use the Change to Cuvette/NanoVolume button below the parameter area (NP80/C40 only).



2. Select protein assay type from drop-down of the parameter area.
   Options are: BCA Assay, Bradford Assay, Lowry Assay, Biuret Assay

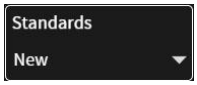
3. **For Cuvette Application:**
   Select the path length depending on the used cuvette.
   Options are: 1 mm, 2 mm, 5 mm and 10 mm
   For NanoVolume Application:
   Select the dilution depending on the sample concentration
   Note: There is no automatic path length setting available in this method.
   Select either a virtual dilution of 15 (path length 0.67 mm) or of 140 (path length 0.07 mm).



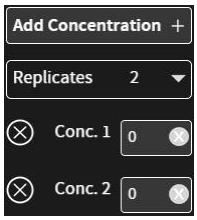
4. If it is desired to heat the sample to 37°C use the toggle switch to turn on cell holder heater. When the cuvette holder has 37°C the color changes to green.
   Note: Only available for cuvette applications (NP80 and C40).

5. The baseline correction is disabled as the default. It is possible to choose between different wavelengths for the baseline correction.
   Options are: 377 nm, 604 nm, 770 nm (not N50) and 823 nm (not N50)

6. Select the curve fit type: Options are linear regression and zero regression (forces the straight line through the origin)



7. Standard new (no selection possible)



New:
Add up to 20 Concentrations and choose Replicates none, 2 or 3.

Measure a blank and depending on the replicate setting all concentrations. Results will be shown in the results area and if replicates are selected a mean value is shown. It is possible to exclude single measurements from the curve calculation by switching the toggle switch off.
Note: The standard curve cannot be altered any longer once the first sample is measured.

8. Once Standard curve is created or loaded it will be used for concentration calculations in the method. It might be necessary to do a blank measurement
Note: Use for NanoVolume measurements always a minimum of 1 µl for any blank measurement.

9. Apply sample and press the sample button to initiate the measurement.
Note: Once the sample measurement is initiated it is not possible to do changes to the standard curve.

CALCULATIONS

Protein concentration is determined using the standard curve by correlating absorbance values of samples with known concentration to calculate the concentration of the unknown sample. In order to maintain accuracy and precision please ensure that the R^2 value of the standard curve is 0.95 or greater.

KINETICS

Please contact the Implen support for further details.

OD600

METHOD OVERVIEW

The growth of bacteria in liquid culture media is commonly monitored by measuring the optical density at 600 nm (OD600) in small samples taken from the cultures. OD600 measurements are typically used to determine the stage of growth of the bacterial culture, thereby ensuring that cells are harvested at an optimum point that corresponds to an appropriate density of live cells. Growth of bacterial cells typically progresses through a series of consecutive phases including: lag, log, stationary and decline (see Figure 1). In general, cells should be harvested towards the end of the log phase using the optical density of the samples to determine when this point has been reached. Since optical density in the case of OD600 measurements results from light scattering rather than light absorption, this value varies depending on the type of bacterial cells in the culture in terms of size and shape. Cells are routinely grown until the absorbance at 600 nm (known as OD 600) reaches approximately 0.4 prior to induction or harvesting. A linear relationship exists between cell number (density) and OD 600 up to an absorbance value of 0.6, approximately.

As mentioned above, for turbid samples such as cell cultures, the absorbance measured is due to light scattering, and not the result of molecular absorption. Since the extent of scattering is affected by the optics of the system (distance between the cell holder and instrument exit slit, monochromator optics, slit geometry, etc.), different spectrophotometer types will tend to give different OD 600 readings for the same turbid sample. Therefore, if results from different spectrophotometers are to be compared, they must be normalized first using appropriate calibration curves.

A calibration curve can be constructed by comparing measured OD 600 to expected OD 600. Expected OD 600 is determined by counting cell number using an alternative technique (for example microscope slide method) and converting to OD 600 using the rule of thumb that 1 OD 600 = 5 x 10^8 cells/ml for *E. coli*.

The NanoPhotometer comes with a correction factor of 1 as default. To compare OD 600 values between different spectrophotometers, it is necessary to determine the constant deviation or ratio between the absorbance values for the same sample from each instrument and use this factor within the setting “correction factor” of your NanoPhotometer Software.

Note: The use of 10 mm path length disposable cells is recommended for optical density measurements of cell culture solutions. Also, to prevent the suspension settling too quickly and giving an OD reading that changes with time, glycerol should be added to the sample.

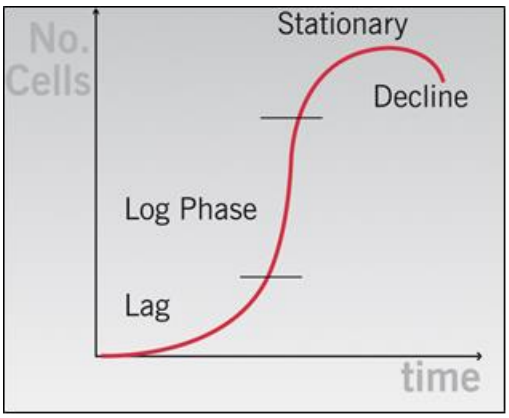


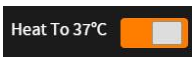
Figure 1 Bacterial growth curve

MEASUREMENTS PROTOCOL

Note: It is not recommended to use the NanoVolume application for the OD600 application. For best results use the cuvette mode (NP80 and C40 models only) for OD600 measurements.

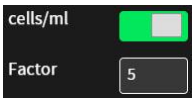
1. Select the OD600 icon on the home screen

2. **For Cuvette Application:**
   Select the path length depending on the used cuvette.
   Options are: 1 mm, 2 mm, 5 mm and 10 mm
   For NanoVolume Application:
   Select the dilution depending on the sample concentration
   Note: There is no automatic path length setting in this method. Select either a virtual dilution of 15 (path length 0.67 mm) or of 140 (path length 0.07 mm)



3. If it is desired to heat the cuvette compartment to 37°C turn the toggle switch on for heated cuvette. Once the cuvette reaches the desired temperature the switch will turn from orange to green.

4. Default wavelength is 600 nm but can be changed, depending on the application.



5. Toggle switch cells/ml is default disabled. Enable cells/ml to get the cells/ml calculated. Enter the cell specific factor and multiplier (e.g. 1 OD600 = 5 x 10^8 cells/ml)

6. Enter the correction factor to compensate for different optical configurations between this and other instruments

7. Option to smooth the graph with different boxcars. off, 1 = boxcar 11 (default), 2 = boxcar 21 and 3 = boxcar 61

8. Option to set/calculate a dilution factor for manual diluted samples.

9. Insert cuvette with the reference sample and select blank button to initiate the measurement
   Note: Use for NanoVolume measurements always a minimum of 1 µl for any blank measurement.

10. Insert cuvette with the sample and select the sample button to initiate the measurement

CALCULATIONS

$OD600 = A_{600} * Đ * cf$

OD600	Optical density at 600 nm
A_{600}	Absorbance at 600 nm (10 mm path)
Đ	Dilution factor
cf	Correction factor for spectrophotometer

$Cells/ml = A_{600} * Đ * cf * multiplier$

A_{600}	Absorbance at 600 nm (10 mm path)
Đ	Dilution factor
cf	Correction factor for spectrophotometer
multiplier	Multiplier of sample

MORE APPS

The More Apps icon located on the home screen opens another menu screen with access to icons for additional applications available on the NanoPhotometer. The applications featured in this menu include: wavelength, concentration, wavescan, absorbance/ratio, standard curve and custom applications.

MORE APPS: WAVELENGTH

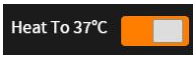
METHOD OVERVIEW

In the wavelength application it is possible to measure simple absorbance (A) and % transmittance (%Trans. / only in cuvette mode) of a sample at specific wavelengths. It is possible to add up to 20 different wavelengths

MEASUREMENT PROTOCOL

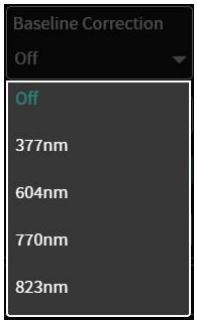
1. Select the More Apps icon from the Home screen and then the Wavelength icon from the More Apps screen

2. **For NanoVolume application:**
   Select the dilution depending on the sample concentration
   Note: There is no automatic path length setting in this method. Select either a virtual dilution of 15 (path length 0.67 mm) or of 140 (path length 0.07 mm)
   For cuvette application:
   Select the path length depending on the used cuvette.
   Options are: 1 mm, 2 mm, 5 mm and 10 mm



3. If it is desired to heat the sample to 37°C use the toggle switch to turn on cell holder heater. When the cuvette holder has 37°C the color changes to green.
   Note: Only available for cuvette applications (NP80 and C40).

4. Enter desired wavelength (λ) to be measured. It is possible to measure up to 20 wavelengths simultaneously. More wavelength (λ) options are added by selecting the wavelength button. The added wavelength can be deleted with ⊗

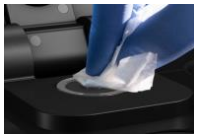


5. Baseline correction is default off and can be enabled by selecting the different values for the correction in the drop-down menu: 377 nm, 604 nm, 770 nm (N/A N50) and 823 nm (N/A N50)

6. Option to smooth the graph with different boxcars. off, 1 = boxcar 11 (default), 2 = boxcar 21 and 3 = boxcar 61

7. Option to set/calculate a dilution factor for manual diluted samples.

8. Apply the blank ddH20 or buffer to the illuminated sample window for the reference measurement and select blank to initiate the reading.
   Note: Use always a minimum of 1 µl for any blank measurement.
   Note: The illumination of the sample window can be switched off in the preferences.



9. Use a lint-free laboratory wipe to clean both the sample window and mirror prior to applying the next sample.
   Note: It could be helpful to apply the blank a second time and read it as a sample to ensure a proper blank.

SAMPLE

10. Apply sample to the sample window and press the sample button to initiate the measurement.

CALCULATIONS

- **Absorbance Calculation:**

Absorbance is formally defined as the decimal logarithm (base 10) of the reciprocal of transmittance:

$$A = \log\left(\frac{1}{T}\right) = -\log T$$

$$T = 10^{(-A)}$$

Note: Corresponding absorbance value e.g. Absorbance value (λ = 230) etc. normalized to 10 mm path length

- **%Transmittance Calculations (cuvette mode only)**

In the wavelength application it is possible to measure the absorbance (A) and % transmittance (%T) on a sample with respect to a reference at a specific wavelength. Transmittance is the ratio of light intensity remaining after it has passed through the sample (*I*) to the initial incident light intensity (I_0):

$$T = \frac{I}{I_0}$$

$$\%T = \frac{I}{I_0} \times 100$$

MORE APPS: WAVESCAN

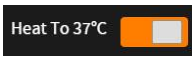
METHOD OVERVIEW

Using the wavescan application it is possible to obtain the complete spectral scan for a defined wavelength range between 200 - 900 nm (NP80/N60/C40) or from 200 - 650 nm (N50).

MEASUREMENT PROTOCOL

1. Select the More Apps icon from the Home screen and then the Wavescan icon from the More Apps screen

2. **For NanoVolume application:**
   Select the dilution depending on the sample concentration
   Note: There is no automatic path length setting in this method. Select either a virtual dilution of 15 (path length 0.67 mm) or of 140 (path length 0.07 mm)
   For cuvette application:
   Select the path length depending on the used cuvette.
   Options are: 1 mm, 2 mm, 5 mm and 10 mm



3. If it is desired to heat the sample to 37°C use the toggle switch to turn on cell holder heater. When the cuvette holder has 37°C the color changes to green.
   Note: Only available for cuvette applications (NP80 and C40).

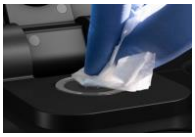
4. Set desired wavelength range (Start and End Wavelength) to be scanned for the samples.
   Note: If samples with a different wavelength range are selected, the graphs are shown on the full scan range of 200 – 900 nm (N50: 200-650 nm)

5. Baseline correction is off as the default and can be enabled by selecting the different values for the correction in the drop-down menu: 377 nm, 604 nm, 770 nm (N/A N50) and 823 nm (N/A N50)

6. Option to smooth the graph with different boxcars. off, 1 = boxcar 11 (default), 2 = boxcar 21 and 3 = boxcar 61

7. Option to set/calculate a dilution factor for manual diluted samples.

8. Apply the blank ddH20 or buffer to the illuminated sample window for the reference measurement and select blank to initiate the reading.
   Note: Use always a minimum of 1 µl for any blank measurement.
   Note: The illumination of the sample window can be switched off in the preferences.



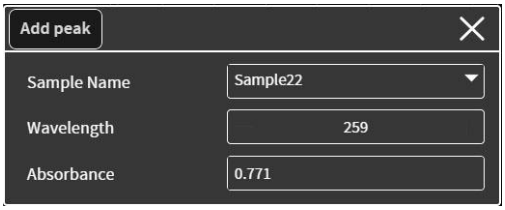
9. Use a lint-free laboratory wipe to clean both the sample window and mirror prior to applying the next sample.
   Note: It could be helpful to apply the blank a second time and read it as a

sample to ensure a proper blank.

10. Apply sample to the sample window and press the sample button to initiate the measurement.

CALCULATIONS

No calculations necessary, values are reported based on 10 mm path length.
In the results are the five prominent peaks shown with wavelength and absorbance value. For cuvette measurements it is possible to change to the %Transmittance mode. If a peak of interest is not shown in the results the peak can be added by clicking on the graph and added to the results by tapping on the Add Peak button in the pop-up window:



MORE APPS: ABSORBANCE RATIO

METHOD OVERVIEW

In this mode, it is possible to determine simple absorbance ratios for a given sample by measuring the absorbance at two wavelengths specified in the parameters of the method relative to a blank.

MEASUREMENT PROTOCOL

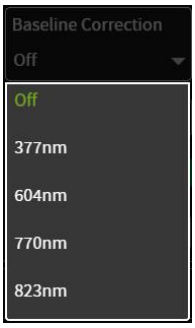
1. Select the More Apps icon from the Home screen and then the Absorbance/Ratio icon from the More Apps screen

2. **For NanoVolume application:**
   Select the dilution depending on the sample concentration
   Note: There is no automatic path length setting in this method. Select either a virtual dilution of 15 (path length 0.67 mm) or of 140 (path length 0.07 mm)
   For cuvette application:
   Select the path length depending on the used cuvette.

Options are: 1 mm, 2 mm, 5 mm and 10 mm

3. If it is desired to heat the sample to 37°C use the toggle switch to turn on cell holder heater. When the cuvette holder has 37°C the color changes to green.
Note: Only available for cuvette applications (NP80 and C40).

4. Enter desired wavelengths (λ 1-1 and λ 1-2) for ratio calculation. It is possible to measure up to 20 absorbance/ratios simultaneously. More wavelength for ratio calculation can be added by selecting the Add Ratio button. The added ratios can be deleted with ⊗

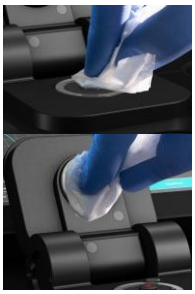


5. Baseline correction is off as the default and can be enabled by selecting the different values for the correction in the drop-down menu: 377 nm, 604 nm, 770 nm (N/A N50) and 823 nm (N/A N50)

6. Option to smooth the graph with different boxcars. off, 1 = boxcar 11 (default), 2 = boxcar 21 and 3 = boxcar 61

7. Option to set/calculate a dilution factor for manual diluted samples.

8. Apply the blank ddH20 or buffer to the illuminated sample window for the reference measurement and select blank to initiate the reading.
Note: Use always a minimum of 1 µl for any blank measurement.
Note: The illumination of the sample window can be switched off in the preferences.



9. Use a lint-free laboratory wipe to clean both the sample window and mirror prior to applying the next sample.
Note: It could be helpful to apply the blank a second time and read it as a sample to ensure a proper blank.

10. Apply sample to the sample window and press the sample button to initiate the measurement.

CALCULATIONS

The absorbance ratio is calculated from the two path lengths specified by the user in the parameters.

$$\lambda_1 : \lambda_2 = \frac{\lambda_1}{\lambda_2}$$

$\lambda_1 : \lambda_2$ = Absorbance Ratio
λ_1 = Absorbance 1 corresponding absorbance value 1 selected normalized to 10 mm path
λ_2 = Absorbance 2 corresponding absorbance value 2 selected normalized to 10 mm path

MORE APPS: CONCENTRATION

METHOD OVERVIEW

In this mode, concentration can be calculated for a sample by determining the absorbance at a specific wavelength relative to a reference. The concentration is then obtained by multiplying the measured absorbance by a specific factor. This factor may be known in advance and entered by the user, or it may be calculated by the instrument by measuring a set of standard (standard curve method) with known concentrations to create a standard curve.

MEASUREMENTS PROTOCOL

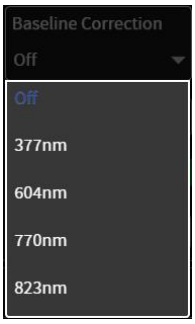
1. Select the More Apps icon from the Home screen and then the Concentration icon from the More Apps screen.

2. **For NanoVolume application:**
   Select the dilution depending on the sample concentration
   Note: There is no automatic path length setting in this method. Select either a virtual dilution of 15 (path length 0.67 mm) or of 140 (path length 0.07 mm)
   For cuvette application:
   Select the path length depending on the used cuvette.
   Options are: 1 mm, 2 mm, 5 mm and 10 mm

3. If it is desired to heat the sample to 37°C use the toggle switch to turn on cell holder heater. When the cuvette holder has 37°C the color changes to green.
   Note: Only available for cuvette applications (NP80 and C40).

4. Default wavelength is 260 nm but can be changed, depending on the application.

5. Enter a factor for concentration calculation

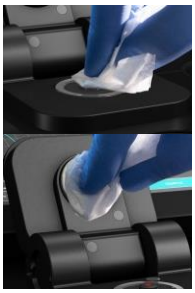


6. Baseline correction is off as the default and can be enabled by selecting the different values for the correction in the drop-down menu: 377 nm, 604 nm, 770 nm (N/A N50) and 823 nm (N/A N50)

7. Option to smooth the graph with different boxcars. off, 1 = boxcar 11 (default), 2 = boxcar 21 and 3 = boxcar 61

8. Option to set/calculate a dilution factor for manual diluted samples.

9. Apply the blank ddH20 or buffer to the illuminated sample window for the reference measurement and select blank to initiate the reading.
Note: Use always a minimum of 1 µl for any blank measurement.
Note: The illumination of the sample window can be switched off in the preferences.



10. Use a lint-free laboratory wipe to clean both the sample window and mirror prior to applying the next sample.
Note: It could be helpful to apply the blank a second time and read it as a sample to ensure a proper blank.

11. Apply sample to the sample window and press the sample button to initiate the measurement.

CALCULATIONS

In this method, the concentration of the sample is calculated based on the Beer-Lambert law given the user specified wavelength of interest and user defined extinction coefficient. The equations for calculating concentration with and without background correction are as follows:

Without background correction:

$$C = A_n * \varepsilon * Đ$$

C	Concentration (ng/µl)
A_n	Absorbance at user specified path length n (10 mm path)
Đ	Dilution factor
ε	extinction coefficient/factor

MORE APPS: STANDARD CURVE

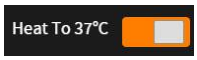
METHOD OVERVIEW

The construction of a calibration curve from multiple standards of known concentrations can be created and stored on the NanoPhotometer. The standard curve can be used to quantify samples of the same type with unknown concentrations. This application provides an extremely useful tool with which to integrate, expedite and simplify the measurement and calculations involved in determining the concentration of analytes in unknown samples. If a zero concentration standard is required, include it in the number of standards to be entered using a reagent blank and entering 0.00 for concentration.

MEASUREMENTS PROTOCOL

1. Select the More Apps icon from the Home screen and then the Standard Curve icon from the More Apps screen.

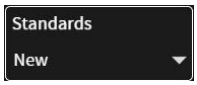
2. **For NanoVolume application:**
   Select the dilution depending on the sample concentration
   Note: There is no automatic path length setting in this method. Select either a virtual dilution of 15 (path length 0.67 mm) or of 140 (path length 0.07 mm)
   For cuvette application:
   Select the path length depending on the used cuvette.



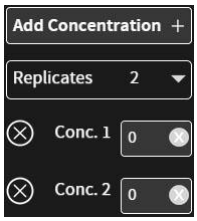
3. If it is desired to heat the sample to 37°C use the toggle switch to turn on cell holder heater. When the cuvette holder has 37°C the color changes to green.
   Note: Only available for cuvette applications (NP80 and C40).

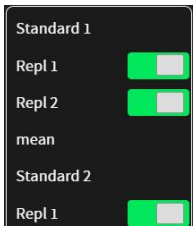
4. The baseline correction is disabled as the default. It is possible to choose between different wavelengths for the baseline correction.
   Options are: 377 nm, 604 nm, 770 nm (N/A N50) and 823 nm (N/A N50)

5. Select the curve fit type: Options are linear regression and zero regression (forces the straight line through the origin)



6. Standard new no selection possible





New:
Add up to 20 Concentrations and choose Replicates none, 2 or 3.

Measure a blank and depending on the replicate setting all concentrations. Absorbances will be shown in the results area and if replicates are selected a mean value is shown. It is possible to exclude single measurements from the curve calculation by switching the toggle switch off.
Note: The standard curve cannot be altered any longer once the first sample is measured.

7. Once Standard curve is created or loaded it will be used for concentration calculations in the method. It might be necessary to do a blank measurement.
Note: Use for NanoVolume measurements always a minimum of 1 µl for any blank measurement.

8. Apply sample and press the sample button to initiate the measurement.
Note: Once the sample measurement is initiated it is not possible to do changes to the standard curve.

CALCULATIONS

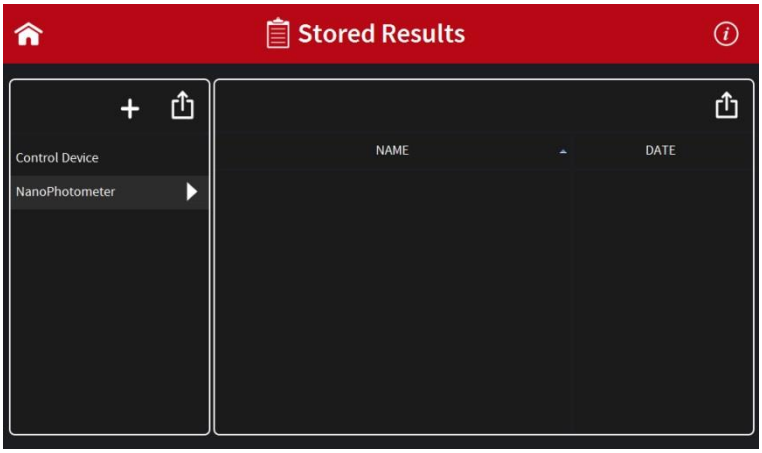
Concentration is determined via the absorbance values provided by the standard curve based on the curve fit selection including the following options: linear regression or zero regression.

CUSTOM APPS

There is an option for designing customer specific Custom Apps which can be loaded to the NanoPhotometer. For more information about designing custom applications to suit individual research needs please contact Implen directly for assistance.

STORED RESULTS

The Stored Results icon opens the directories of folders containing files of results that have been previously saved.



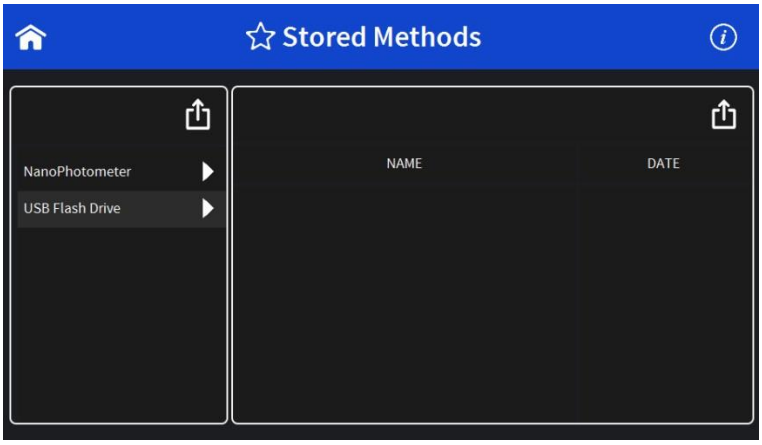
On the left side of the screen all available directories/folders are shown: NanoPhotometer, Control Device and/or USB flash drive. To open a folder click on the ▸ icon, for selecting the folder click on the folder name.
Folder can be created by tapping on +. Folders can be deleted, renamed, moved or copied to by clicking on the ⍐ icon.

On the right side of the screen all saved result files of the selected folder are shown and can be opened by a long or double click.
It is also possible to delete, rename, move or copy files by clicking on the ⍐ icon.

Note: Control device is only available on tablets and smartphones not on the NanoPhotometer and computer versions of the software.

STORED METHODS

The stored methods icon opens the directories of folders containing methods stored by the user.



On the left side of the screen all available directories are shown: NanoPhotometer and/or USB flash drive. To open a folder click on the ▸ icon, for selecting the folder click on the folder name. New folders can be created by clicking on +. Folders can be deleted, renamed, moved or copied to by clicking on the ⍐ icon.

On the right side of the screen all saved methods of the selected folder are shown and can be opened by a long or double click.
It is also possible to delete, rename, move or copy methods by clicking on the ⍐ icon.

5. PREFERENCES

System preferences can be set by selecting preferences (⚙) on the home screen. The preferences menu includes: general, dyes and network. The menu item which is selected will have its preference options listed in the window to the right.

Note: Preferences are not available for smart phone < 7" screen versions.

GENERAL

Selecting General in the Preferences menu opens a window to the right of the preferences menu with the following options: Date & Time, Region, Display (built-in display only), Statistics, About and Illumination Sample Window.

DISPLAY

The brightness of the built-in screen can be adjusted under display.

Note: Display preferences are only available for instruments with integrated touchscreen.

ABOUT

In about the following information about the NanoPhotometer are shown: NanoPhotometer model, serial number, IP address, hardware version, firmware version and software.

WIFI ACCESS POINT

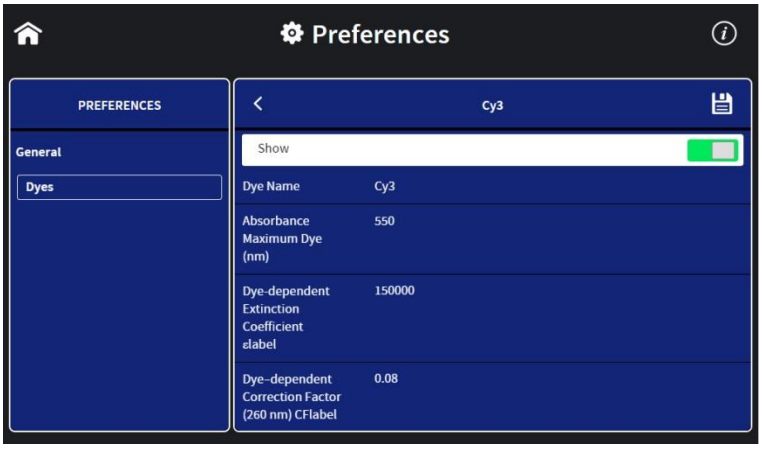
Toggle Switch to en-/disable the WiFi access point.

ILLUMINATION SAMPLE WINDOW

Toggle switch to switch on/off the illumination of the sample window.
Note: Only available for NP80/N60/N50 models.

DYES

There is a list of available dye-labels for both nucleic acid dye and protein dye methods. Each dye has either an (🔒) icon in front of the dye name indicating that the dye is locked and cannot be changed or deleted. If the dye has a delete symbol (🗑), there is an option to delete the dye which is not locked or pre-programmed into the NanoPhotometer.
Selecting a dye name opens a new screen with the dye information: dye name, absorbance maximum dye (nm), dye-dependent extinction coefficient ε_{dye} ($M^{-1} * cm^{-1}$), and dye-dependent correction factor as well as the option to show the dye in the drop-down of the application (Nucleic Acid or Protein UV).



Note: It is not possible to delete a dye of the factory list; custom dyes can be deleted if they are not locked.

It is possible to add a new dye to the list by selecting the + button to add a new dye. A window will open where it is possible to enter the: dye name, dye absorbance maximum (nm), dye-dependent extinction coefficient ε_{dye} ($M^{-1} * cm^{-1}$), and dye-dependent correction factor. There is a toggle switch available to lock the dye to prevent deleting a dye from the dye list accidentally.

6. TROUBLESHOOTING

SELF-CALIBRATION TEST

The NanoPhotometer self-calibration test is performed automatically every time the instrument is powered on. If the instrument passes the self-calibration test the home screen is shown. If the instrument does not pass the test, a window with a message will appear along with the message explaining the reason for the failed test along with the recommended solution. If the ok button is selected, the window is closed and the home screen will be shown with an error message at the top. The error message is also shown on top of all other screen and all performed measurements including the saved files of those measurements. If the self-calibration test fails please contact the Implen Support Team.

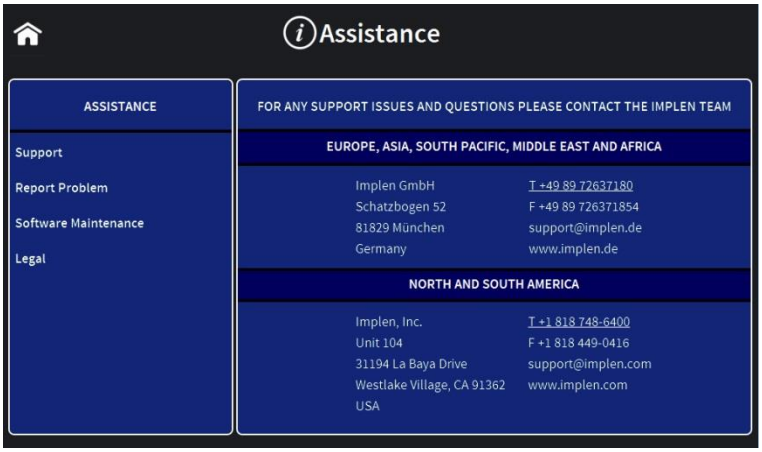
7. ASSISTANCE

The Assistance menu includes: support, report a problem (only available for tablet and computer versions), software maintenance and legal as functions to help with any technical issues or questions that may arise with the NanoPhotometer. The menu list item selected on the left side assistance menu will appear in the window to the right.

Note: Assistance function is not available for smart phone < 7” screen versions.

SUPPORT

Selecting support on the left side assistance menu will show the available options for contacting Implen.



REPORT PROBLEM

The function to report a problem is available on computer and tablet versions only. When report a problem is highlighted in the left side assistance menu, the right side will have a form to fill out with information including: first name, last name, phone number, email, and country. A drop-down menu provides the option to select the problem type and includes the following selections: error message, software, firmware, measurements, and other. It is also possible to enter a question or comment at the end of the form. Once the form is completed and the send button is selected a message will be sent directly to Implen and the appropriate support person will contact as soon as possible to provide further support.

Note: The report a problem function is only available for computer and tablet versions.

SOFTWARE MAINTENANCE

RESET

There is an option to reset the instrument to factory settings. By selecting the reset button a window will open that says “Would you like to reset the NanoPhotometer?” Selecting the cancel button will close the window without changing the settings and selecting reset will open a window that will ask again” Would you really like to reset the NanoPhotometer to factory settings?” If it is confirmed the factory settings will be restored.

Note: All stored methods, settings and data on the NanoPhotometer will be deleted if the reset option is executed.

SOFTWARE UPDATE

There is a button for software update. It is needed to plug in a USB flash drive with the software installation file to the USB port. The software update files are available on the Implen homepage: www.implen.de.
Update procedure:
1. Copy the NPOS.bin file to a USB flash drive
2. Plug in the USB flash drive to the USB port of the NanoPhotometer
3. Go to Assistance/Software Maintenance
4. Press the Update button. The update will start and once finished automatically reboot the NanoPhotometer.

Note: Make sure that the NanoPhotometer is connected to power and that the power connection is not interrupted during the update.

CONTACT IMPLEN

There is an option to contact Implen for instruments connected to the internet. For any support issues and questions please contact the Implen team directly:

Europe, Asia, South Pacific, Middle East and Africa
Implen GmbH
Schatzbogen 52
81829 München
Germany

Phone: +49 89 72637180
Fax +49 89 726371854
Email: support@implen.de
www.implen.de

North and South America
Implen, Inc.
Unit 104
31194 La Baya Drive
Westlake Village, CA 91362
USA

Phone +1 818 748-6400
Telefax +1 818 449-0416
Email: support@implen.com
Website www.implen.com

8. MAINTENANCE

MAINTENANCE FREE TECHNOLOGY

The NanoPhotometer technology is maintenance-free. Regular maintenance and calibration is not necessary.

For facilities that are working according to national as well as international guidelines and standards including: Good Laboratory Practice (GLP), Good Manufacturing Practice (GMP), or ISO9000-9004; the proper performance of the spectrophotometer has to be tested and proven on a regular basis with individually set intervals. Implen provides certified NanoPhotometer secondary standards as an optional accessory. These NanoPhotometer Didymium glass filters and standard solution sets are suitable for the control and documentation of the wavelength accuracy and the photometric accuracy of your system. IQ/OQ documentation is also available. Please contact your local Implen office or an authorized Implen partner for further information.

Support agreements that help to fulfill the demands of regulatory guidelines concerning GLP/GMP include: calibration certification using filters traceable to international standards (during production and quality control), certified engineers and calibrated test equipment, approved to ISO 9001 standard, automatic self-diagnostic calibration test during start of the NanoPhotometer, result is documented in each data output file, and possibility to save a Implen Document Source (IDS) file (no data manipulation possible).

REPLACEMENT PARTS

- **Lamp Replacement**

The NanoPhotometer is equipped with a xenon flash lamp and should not need replacement until several years of use. In the unlikely event the lamp does need to be replaced, this should be done by the manufacturer or a certified service engineer from your supplier.

- **Battery Replacement**

The optional battery pack can only be assembled or replaced by the manufacturer or a certified service engineer from your supplier.
Note: Danger of explosion if battery is incorrectly replaced. Replacement only with the same or equivalent type recommended by the manufacturer: Replacement needs to be done by a certified service engineer

- **Touchscreen Replacement**

The optional touchscreen can only be assembled or replaced by the manufacturer or a certified service engineer from your supplier.

CLEANING AND GENERAL CARE

Switch off the NanoPhotometer and disconnect the power cord prior to external cleaning. Use a soft wet cloth or dry microfiber cloth to clean all external surfaces. A mild liquid detergent may be used to remove stubborn marks.

Approved disinfectant solutions include: Apesin disinfection spray (Tana Chemi GmbH), Incidin Liquid & Inciddin Foam (Ecolab), and Lysoformin Spezial (Lysoform Dr. Hans Roseman GmbH).

Note: Observe all necessary precautions if dealing with hazardous samples or solvents.

9. WARRANTY

Implen guarantees that the product supplied has been thoroughly tested to ensure that it meets its published specification. The warranty included in the conditions of supply is valid for 12 months and is valid only if the product has been used according to the instructions supplied. Implen or your supplier can accept no liability of loss or damage arising from the faulty or incorrect use of this product

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